Bark and Charcoal Filters for Greywater Treatment

Pollutant Removal and Recycling Opportunities

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Abstract

Water scarcity, inappropriate sanitation and wastewater pollution are critically important global issues. Greywater is a sustainable water source for recycling, so this thesis examined simple, robust, low-cost alternatives for on-site treatment of greywater to irrigation water quality. Laboratory-scale pine bark, activated charcoal and sand filters were evaluated as regards their pollutant removal and interactions between medium properties, greywater, microbial activity and bacterial community structure. The effects of hydraulic and organic loading rates (HLR and OLR) were described by general regression models (GRM). The quality of the treated greywater was evaluated against Jordanian standards for irrigation water. A series of experiments examined treatment of artificial greywater in terms of lowering biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), phosphorus (Tot-P), nitrogen (Tot-N) and pathogen indicators (total thermotolerant coliforms) and tracer microorganisms (enterohaemorrhagic Escherichia coli (EHEC) and bacteriophage PhiX). Following greywater loading, all filter materials developed biofilms with high bacterial diversity and richness. The driving force shaping bacterial communities in bark material was its organic composition and low pH, while the communities in the charcoal and sand filters were more influenced by the greywater. The GRM indicated that the performance of all filters was influenced by the HLR and OLR of the present and previous runs. The organic matter content and surface and hydraulic properties of the bark filters resulted in high BOD_5 removal rates (94-99%), even at increased HLR and OLR, but accompanied by release of dissolved organic substances originating from the bark itself. High nitrification occurred in the bark filters in all loading regimes tested, but with low Tot-N removal. The bark filters demonstrated 1-3 log_{10} removal of microorganisms, but bark organic nature made its filters more vulnerable to biodegradation and disintegration. The charcoal had large specific surface area, which provided the capacity for intermediate-high removal of BOD_5 (83-97%), Tot-N (50-98%) and Tot-P (64-98%), but removal of microorganisms was poor. The sand filters demonstrated low BOD_5 removal (67-91%) and high nitrification, but low nitrogen removal. Greywater treatment by bark and charcoal filters reduced their organics content to acceptable irrigation levels. Nitrogen and microorganisms must be further reduced to meet Jordanian standards on treated wastewater for irrigation.

Keywords: bacterial diversity, biofilm, BOD_5, COD, hydraulic loading, irrigation, nitrogen, organic loading, organic matter, respiration.

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Dedication

To the microbiologist who dedicated his PhD thesis to civil engineers who truly want to learn some microbiology.

Content

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Dalahmeh, S.S., Hylander, L.D., Vinnerås, B., Pell, M., Öborn, I., Jönsson, H. (2011). Potential of organic filter materials for treating greywater to achieve irrigation quality: A review. *Water Science and Technology* **63**(9), 1832-1840.

II Dalahmeh, S., Pell, M., Vinnerås, B., Hylander, L., Öborn, I., Jönsson, H. (2012). Efficiency of bark, activated charcoal, foam and sand filters in reducing pollutants from greywater. *Water, Air, & Soil Pollution* **223**(7), 3657-3671.

III Dalahmeh, S.S., Jönsson, H., Hylander, L., Hui, N., Pell, M. Dynamics and functions of bacterial communities in bark, charcoal and sand filters treating greywater (manuscript).

IV Dalahmeh, S.S, Pell, M., Hylander, L., Lalander, C., Vinnerås, B., Jönsson, H. Greywater treatment using bark, charcoal and sand filters - response to changing hydraulic and organic loading rates (manuscript).

V Lalander, C., Dalahmeh, S., Vinnerås, B., Jönsson, H (2013). Hygienic quality of artificial greywater subjected to aerobic treatment - a comparison of three filter media at increasing organic loading rates *Environmenal Technology,* DOI:10.1080/09593330.2013.783603.

Papers I, II and V are reproduced with the kind permission of the publishers.

The contribution of Sahar Dalahmeh to the papers included in this thesis was as follows:

I Participated in planning the work, conducted the literature review and had the main responsibility for writing the manuscript.

II Participated in planning the experiment and conducted the laboratory work. Interpreted the data together with the co-authors. Had the main responsibility for writing the manuscript, including production of the illustrations.

III Contributed to planning the experiment and conducted all the laboratory work except the DNA sequencing and preparation of the gene library. Interpreted the data together with the co-authors and had the main responsibility for writing the manuscript, including production of the illustrations.

IV Participated in planning the experiment and conducted the laboratory work. Interpreted the data together with the co-authors. Had the main responsibility for writing the manuscript, including production of the illustrations.

V Conducted the chemical analysis, wrote part of the methods section and participated in revisions of the manuscript.

Abbreviations

ANOVA	Analysis of variance
BOD_5	Biochemical oxygen demand
CEC	Cation exchange capacity
COD	Chemical oxygen demand
DNA	Deoxyribonucleic acid
D_{10}	Particle size at which 10% of the material is finer
D_{60}	Particle size at which 60% of the media is finer
E. coli	*Escherichia coli*
EHEC	Enterohaemorrhagic *Escherichia coli*
GRM	General regression models
HLR	Hydraulic loading rate
HLR_i	Hydraulic loading rate during present measuring run
HLR_{i-1}	Hydraulic loading rate during previous measuring run
MBAS	Methylene blue active substances
MENA	Middle East and North Africa
NMS	Non-metric multidimensional scaling
NH_4^+-N	Ammonium nitrogen
NO_3^--N	Nitrate nitrogen
OLR	Organic loading rate
OLR_i	Organic loading rate during present measuring run
OLR_{i-1}	Organic loading rate during previous measuring run
OTU	Operational taxonomic units
PAST	Palaeontological statistics
PTC	Potential treatment capacity
TOC	Total organic carbon
Tot-N	Total nitrogen
Tot-P	Total phosphorus
TSS	Total suspended solids
TTC	Total thermotolerant coliforms

1 Introduction and outline

1.1 Introduction – water scarcity and wastewater pollution

Water is a basic need not only for human survival but also for socio-economic development. Globally, water use increased six-fold during the twentieth century and by the year 2025 about 1.8 billion people will live under absolute water scarcity conditions, *i.e.* with an annual water supply of less than 500 m^3 per capita, and two-thirds of the world's population will experience water stress, *i.e.* will have an annual water supply of less than 1700 m^3 per capita (UN-Water, 2006). By 2030, the planet will host 8 billion people (World Bank, 2010), with the highest growth expected in developing countries, where 82% of the world's population already lives and experiences water scarcity. As a consequence, the demand for water will increase in these countries and water availability for irrigation will be a limiting factor for food production (FAO, 2007).

Freshwater scarcity is a reality in many regions of the world today, *e.g.* the Middle East and North Africa (MENA). While MENA is home to more than 5% of the world's population, only 1% of the globally accessible water is available in this region (Redwood, 2010). The annual per capita water share in MENA is about 1200 m^3, while the global average is 7000 m^3. Considering the population growth and climate change, the annual per capita water share in MENA will decrease to 500-750 m^3 by 2050 (Immerzeel *et al.*, 2011).

No matter how scarce or abundant fresh water is, it is used for daily household activities and after this it becomes wastewater. Household wastewater is composed of blackwater and greywater. Blackwater is the wastewater coming from toilets (faeces, urine, possible toilet paper and flushwater). Greywater is all household wastewater excluding blackwater. This means that greywater includes water from bathtubs, showers, hand basins, kitchen sinks and laundry.

Wastewater management is an issue of concern. Worldwide, 2.5 billion people (37% of the world's population) do not have access to adequate sanitation (WHO/UNICEF, 2012). The inadequate provision of sanitation and wastewater disposal facilities leads to environmental and public health problems and 1.8 million people die every year from diarrheal diseases (Corcoran *et al.*, 2010; WHO, 2008).

Centralised wastewater systems, transporting wastewater to a central wastewater treatment plant, are common in industrialised countries and in some large cities in less industrialised countries. Centralised systems require complex infrastructure and are expensive to construct and operate. In low and middle income countries, lack of expertise, limited financial resources and prioritisation of water supply over sanitation lead to essentially no wastewater receiving treatment before being disposed of (Scott *et al.*, 2004; WHO, 2003) or reused (Jimenez *et al.*, 2010).

Treating wastewater properly before disposal or reuse is not a luxury but a necessity to protect public health, the environment and water resources. One way to achieve proper treatment can be by source separation, local treatment and utilisation of wastewater fractions (urine, faeces and greywater) for crop production. The World Health Organization Guidelines for safe use of wastewater, excreta and greywater highlight the reliability of greywater in alleviating water scarcity, especially for irrigation (WHO, 2006). The reliability of greywater stems from the fact that it is a constant water source, contains plant nutrients and has a comparatively low concentration of pathogens compared with mixed wastewater and blackwater.

Greywater treatment using filters of various carrier materials with different filter pore sizes is a common approach for cleaning greywater. Macropore filters, including simple strainer and mesh filters (Christova-Boal *et al.*, 1996), nylon sock filters (March *et al.*, 2004), gravel filters (Al-Hamaiedeh & Bino, 2010) and sand filters (Suleiman *et al.*, 2010; Friedler & Hadari, 2006), have been used for greywater treatment. Macropore size ranges from 1-5 mm for coarse pores and 0.075-1 mm for fine pores (Beven & Germann, 1982). Generally, coarse macropore filters retain particles and hair, but are not a complete barrier against suspended pollutants, and hence the turbidity, chemical properties and organic loading of the influent are only marginally lowered, which may lead to biological growth in recycling system (Christova-Boal *et al.*, 1996). Besides the effluent quality, frequent cleaning and replacement requirement pose other obstacles with filters relying only on straining. Micro and ultra membrane filters, with pore size 0.1 μm, have been used for greywater treatment (Ramona *et al.*, 2004; Ahn *et al.*, 1998). Microfiltration and ultrafiltration purify greywater to unrestricted use standards

(Ramona *et al.*, 2004). However, fouling and energy consumption are issues of concern (Nghiem *et al.*, 2006), which makes membrane technology economically unfeasible at present for greywater treatment (Friedler & Hadari, 2006), at least not in small-scale applications in low and middle income countries.

Sand filters, which are within the fine macropore range, are the most commonly applied filters for on-site treatment of greywater (Burnat & Eshtaya, 2010; Suleiman *et al.*, 2010; Friedler & Hadari, 2006) and domestic wastewater (EPA, 2002; Pell, 1991). Besides the physical filtration through the sand, an active biofilm develops. It is attached to the sand particle surfaces and mineralize organic matter from the wastewater (Rodgers *et al.*, 2005). Clogging of sand filters is a major problem (Spychała & Błazejewski, 2003). The high bulk density of the sand means large efforts are required for transporting virgin sand and recycling or disposing of spent sand.

While having the same particle size and uniformity coefficient as sand, pine bark and charcoal are distinguished by larger specific surface area, higher porosity, lower density and higher organic content than sand (**Paper II**). Bark and charcoal have been shown to have a large capacity for adsorption of heavy metals (Argun *et al.*, 2009; Babel, 2004) and organic compounds (Li *et al.*, 2010; Mukherjee *et al.*, 2007; Haussard *et al.*, 2003; Ratola *et al.*, 2003). These properties of bark and charcoal are believed to indicate high capacity for greywater treatment, with lower risk of clogging and easy transportation, which needs to be confirmed experimentally.

1.2 Objectives

The overarching aim of this thesis was to provide detailed information on the performance of simple, robust and low-cost alternatives for on-site treatment of greywater. The main research aim was to determine the potential of pine bark (bark), activated charcoal (charcoal) and sand (sand) filters for removal of organic pollutants and pathogens in order to yield water suitable for irrigation, considering also nitrogen and phosphorus. Specific objectives were to:

- Assess the need for treatment of greywater prior to irrigation.
- Investigate the treatment capacity of bark, charcoal and sand filters in removal of organic matter, nitrogen, phosphorus and, indicator and tracer microorganisms in relation to their properties.
- Describe microbial activity, bacterial diversity and composition in the filters and their effects on organic matter reduction and nitrogen transformation.

- Analyse the response of the filters to variable hydraulic and organic loading regimes, in terms of removal capacity of organic matter, nitrogen, phosphorus and microorganisms in bark, charcoal and sand filters.
- Explore the recycling opportunities and constraints of the treated greywater and the spent medium.

The work to achieve these specific objectives is reported in **Papers I-V** and the results are combined and discussed in this thesis in the following five chapters: (Ch. 2) Greywater pollution and treatment requirement for irrigation (**Paper I**); (Ch. 3) Effects of medium properties (**Papers II**, **IV** and **V**); (Ch. 4) Microbial activity, diversity and composition (**Papers II**, **III** and **IV**); (Ch. 5) Loading conditions and treatment capacity (**Papers I**, **II**, **IV** and **V**); (Ch. 6) Recycling opportunities and constraints (**Papers II**, **IV** and **V**). Some general conclusions and perspectives are presented in Ch. 7.

1.3 Strategy of the study

The thesis work started with a literature review to identify the problematic components of greywater and its impact on soil, plant and health, and hence to assess the need for greywater treatment prior to its use for irrigation (**Paper I**). The review included investigation of the potential of organic and agricultural by-products, among them bark, as a potential filter medium for greywater treatment (**Paper I**). Based on the findings in **Paper I**, a laboratory experiment was carried out to test the treatment performance of bark, charcoal, foam and sand filters under controlled conditions. For this, column filters (20 cm diameter and 60 cm deep) consisting of bark, charcoal, foam and sand were prepared (**Paper II**). Prior to being packed into the vertical columns the materials, except the foam, were sieved to all have similar effective particle size distribution (Figure 1). The different media were characterised for pH, loss on ignition, particle density and specific surface area according to the methods reported in **Paper II**. The filters were also characterised for porosity, hydraulic conductivity and hydraulic residence time (**Paper II**). The performance of the foam medium proved inferior to that of the other materials, and it was therefore omitted from the subsequent studies.

The hydraulic properties and specific surface properties of bark, charcoal and sand all differed. In order to understand the influence of medium properties on the physical-chemical treatment capacity, as presented in **Paper II**, and the biofilm and biological activity development, as presented in **Paper III**, the performance of the filters in terms of pollutant removal was studied at constant hydraulic loading rate (HLR) of 32 L m^{-2} day^{-1} and an organic loading rate (OLR) of 14 g BOD_5 m^{-2} day^{-1}. For the purposes of analysing pollutants removal from greywater during the start-up and initial steady state operation, influent and effluent water samples were collected and total suspended solids (TSS), biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total organic carbon (TOC), methylene blue active substances (MBAS), ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), total nitrogen (Tot-N), phosphate (PO_4-P), total phosphorus (Tot-P), total thermotolerant coliforms (TTC) and *Enterococcus* spp. were analysed (**Paper II)**. For the purposes of evaluating differences in potential biofilm performance and microbial community structure between bark, charcoal and sand filters, samples of filter media were collected from 0, 20, 40 and 60 cm depth in each filter every two weeks and analysed for respiration activity and bacterial composition by 16S rRNA genes followed by 545 pyrosequencing (**Paper III**).

As greywater flows and composition vary daily, weekly and seasonally, and sometimes even cease completely, *e.g.* in summer cottages. Filters for on-site greywater treatment should integrate the composition and loading patterns of the expected greywater with the properties of the medium used. To achieve this, the effects of variable hydraulic and organic loading on BOD_5, COD, Tot-N and Tot-P removal (**Paper IV**), as well as removal of TTC and added tracer enterohaemorrhagic *Escherichia coli* (EHEH) and the bacteriophage PhiX 174 (**Paper V**), were studied. The filters were fed with artificial greywater, first with increasing HLR of 32-128 L m^{-2} day^{-1} and then with increasing OLR of 14-76 g BOD_5 m^{-2} day^{-1}.

Artificial greywater was used in all experiments (**Papers II-V**). The artificial greywater was mixed with 4% (v/v) real wastewater to inoculate it with bacterial flora and insure presence of bacterial populations similar to these found in real greywater. The artificial greywater used covered a wide range of levels of organic matter, nitrogen and phosphorus . These levels can be found in natural greywater under different circumstances (Table 1). The quality of the artificial water agreed well with greywater from low (Halalsheh, 2008; Bongumusa, 2007), medium (Morel & Diener, 2006) and high water consumption settings (Vinnerås *et al.*, 2006). Most of the pollutants in the artificial greywater were in dissolved form, and therefore the suspended solids content was low (Table 1). Low total suspended solids (TSS) content can occur in natural greywater, which according to Jefferson *et al.* (2000) has organic strength comparable with wastewater, but has relatively low suspended solids. This greywater also resembles greywater primarily treated in a settling tank. EHEC and PhiX phage are usually not found in greywater. However, outbreaks of EHEC might result in high bacterial numbers in greywater due to faecal contamination, handling of contaminated food, showers and anal cleaning of infected individuals. The PhiX phage, which usually infects *E. coli*, was used as a model for any virus, including human viruses, that can be found in greywater.

In all experiments the greywater feed was kept at 25 °C and the external temperature varied between 25 and 29 °C, resembling the ambient temperature in warm climate settings. The greywater was fed intermittently in three doses per day, at proportions of 70, 10 and 20% of the daily HLR, based on a hydrograph for greywater generation in a typical household in a rural community in Jordan (Ghunmi *et al.*, 2008). The filters were allowed to rest for eight months between the two main studies (**Papers II** and **IV**).

Analysis of variance (ANOVA) at 95% confidence level was used to assess differences in pollutant reduction between the filter materials. Multiple linear regression was used to assess and describe the influence of HLR and OLR on the reduction in pollutants. The models were built step-wise using general regression models (GRM). Non-metric multidimensional scaling (NMS) using palaeontological statistics (PAST) was used to evaluate differences in microbial composition between the filters.

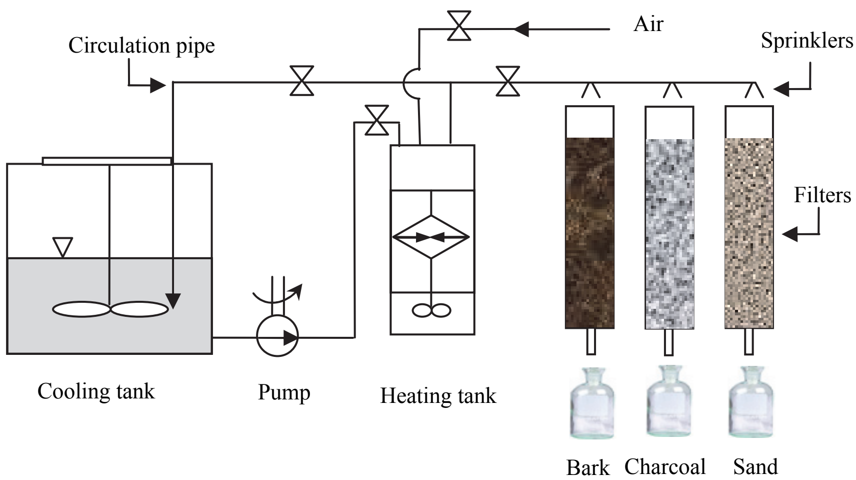

Figure 1. Schematic diagram of the greywater filter system used in all experiments (adapted from **Paper II**).

2 Greywater pollution and treatment requirement for irrigation

2.1 Greywater pollution

Greywater accounts for 60-70% of total wastewater in households with a flush toilet (Friedler & Hadari, 2006), with daily generation rates usually ranging from 30 to 120 L capita^{-1} in low and middle income countries (Morel & Diener, 2006). The concentrations of salts, solids, organic matter, nitrogen, phosphorus and pathogens in greywater vary widely (Christova-Boal *et al.*, 1996) depending on *e.g.* country, location, personal habits and cleaning products used in the home.

The organic matter content in greywater ranges between 13 and 8000 mg COD L^{-1} (Eriksson, 2002). The organic material originates from detergent, food, dirt and skin residues and is highly degradable under both aerobic (84%) and anaerobic (72%) conditions (Zeeman *et al.*, 2008). The nitrogen content can range from 0.6 to 74 mg L^{-1}, as reviewed by Eriksson *et al*. (2002), which is low compared with household wastewater. Greywater with high nitrogen concentrations has also been reported (Halalsheh, 2008). The phosphorus level depends on whether phosphorus-containing detergents are used, or prohibited by local or regional regulations. Phosphorus levels are within 4-14 mg P L^{-1} in non-phosphorus detergent greywater and 6-23 mg P L^{-1} in areas where phosphorus-containing detergents are still in use (Eriksson *et al*., 2002). Greywater can be contaminated with *E. coli*, *Enterococcus*, *Salmonella* and enteropathogenic *E. coli* (EPEC*)* (O'Toole *et al.*, 2012; Ottoson & Stenstrom, 2003; Rose, 1991) and enteric viruses such as norovirus and rotavirus (O'Toole *et al.*, 2012). Faecal contamination is the major source of *E. coli* and *Enterococcus* (Christova-Boal *et al*., 1996), while *Salmonella* can be introduced into kitchen wastewater during food handling (WHO, 2006).

2.2 Greywater for irrigation

While greywater is a reliable source for irrigation, its use without treatment may pose hazards to the environment and to public health because of its content of organic and chemical compounds, as well as pathogens. Although the organic matter content in greywater adds to the soil organic matter content, excessive release of untreated greywater including fat and surfactants has been reported to negatively affect soil and plants (Travis *et al.*, 2010; Shafran, 2006; Abu-Zreig *et al.*, 2003), causing hydrophobic soil phenomena (**Paper I**; Shafran *et al*. 2006), plant toxicity (**Paper I**; Shafran *et al*., 2006) and production of malodorous compounds (Eriksson, 2002). In hydrophobic soils, the water retention capacity is low, and thus the water availability for plants is restricted, causing wilting effects. Blockage of irrigation pipes is another operating problem caused by solids, organics and fat in greywater (**Paper I**). Moreover, irrigation might not occur every day, and thus greywater storage might be needed. Storage of untreated greywater is aesthetically disturbing due to development of malodorous compounds (**Paper I**). Storing greywater for 48 hours causes rapid degradation of the organic material, accompanied by generation of offensive odours, growth of indicator bacteria, survival of *Salmonella* and *Shigella* and mosquito breeding (WHO, 2006; Dixon *et al.*, 2000; Christova-Boal *et al.*, 1996; Rose, 1991). Health threats due to potential pathogen contamination are a major concern. The microbial health risks of greywater may arise upon use (Rose *et al*., 1991) either by direct physical contact or indirectly by transmission of pathogens from irrigated crops to consumers (WHO, 2006). While nitrogen and phosphorus are considered valuable for fertilisation, if greywater has high content of these the excess nutrients can leach into the environment and cause eutrophication or groundwater contamination. Regulations for wastewater disposal and recycling are set to protect the environment and public health, which means that low levels of organics, nutrients and indicator organisms are required in greywater intended for recycling (Table 1).

In conclusion, greywater with high concentrations of organic matter and pathogenic indicators requires treatment to avoid risk to environmental and health threats, and to comply with disposal and recycling regulations.

Table 1. *Quality of the artificial greywater used in this thesis, greywater quality values reported in the literature and criteria for recycling of wastewater for irrigation in different countries*

Parameter	Artificial greywater	Greywater	USA-EPA	Jordan	EU	Italy	Australia
Recycling option			Restricted irrigation	Cooked vegetables	Discharge from urban wastewater treatment facilities	Irrigation	Surface irrigation
pH	6.9-8.3	6.3-8.35[a]	6-9	6-9		6-9.5	
SS (mg L^{-1})	113	76-1396 [a]		50	35	10	30
BOD_5 (mg L^{-1})	126-1390	129-2287 [a]	10	30	25	20	20
COD (mg L^{-1})	420-4800	13-8000[b]		100	125	100	
Tot-N (mg L^{-1})	25-241	25-211 [ac]		45	15	15	
Tot-P (mg L^{-1})	1.7-10.2	2.4-27 [ac]		30	1-2	2	
E.coli (MPN $100mL^{-1}$)		2×10^{5} [c]		100		10	10
FC (CFU $100mL^{-1}$)	TTC: $4.30x10^4$-$6.09x10^6$- EHEC: $1.0x10^8$-$4.3x10^8$ PhiX: $2.7x10^6$-$8.1x10^6$ PFU $100mL^{-1}$	10^4-10^8 [a]	0				
Source	**Papers II-V**		USEPA (2004)	JISM (2006)	The Council of the European Communities (1991)	Chaillou *et al.* (2011)	Chaillou *et al.* (2011)

[a] Morel & Diener (2006)
[b] Eriksson *et al.* (2009)
[c] Halalsheh (2008)

3 Effects of filter medium properties

Filter medium acts as a substrate for physical filtration, physico-chemical adsorption and microbial growth. Medium properties such as particle size and distribution, specific surface area, surface chemical composition and porosity affect the removal capacity of pollutants. Adsorption, which is known to remove organic matter, NH_4^+-N, PO_4-P and bacteria, is influenced by grain size and surface characteristics of the medium (Stevik *et al.*, 1999). Grain size determines the specific surface area available for adsorption and biofilm development (Moore *et al.*, 2001). Mineral composition and chemical functional groups on the surface determine the charge and activity of the surface, which in turn determine the type of pollutants that can be adsorbed (Amonette & Josheph, 2009; Siegrist *et al.*, 2000). In addition, adsorption is a prerequisite for microbial attachment (Trulear & Characklis, 1982), where the medium hosts the microbes, and the adsorbed organic matter and nutrients provide feed (Li & DiGiano, 1983). Porosity, which is the amount of voids available in the filter, determines water retention, hydraulic conductivity and hydraulic residence time in the filters. These factors affect both adsorption and biofilm activities, which in turn affect the retention of solids and bacteria and transformation of organic matter, nitrogen and phosphorus. In the following sections, the effects of specific surface and hydraulic properties of the bark, charcoal and sand filter media tested in this thesis on organic matter, solids, nitrogen, phosphorus and indicator and tracer microorganisms removal are discussed, based on results from **Papers II, IV** and **V**.

3.1 Effects of surface properties on pollutant removal capacity

The specific surface area of the pine bark used in the experiments was 0.734 m^2 g^{-1} (Table 2). In the bark filters, 44% of sodium chloride (NaCl) added during a hydraulic retention experiment was not recovered, but sorbed by the medium (**Paper II**) and high removal of BOD_5, MBAS, NH_4^+-N, TTC, EHEC and PhiX was achieved (Table 3; **Papers II, IV** and **V**). The effluent from the bark filters had lower pH (5.6-6.3) than the effluent from the charcoal (6.5-7.8) and sand filters (6.6-7.7). Moreover, the effluent from bark had a yellow tint and high COD content (200-590 mg L^{-1}) (**Papers II** and **IV**).

Table 2. *Characteristics of the bark, charcoal and sand filter materials used in the study*

Parameter	Bark	Charcoal	Sand
pH (SU)	5.1	10.4	7.9
Loss on ignition (%)	90	90	<1
Effective size (mm)	1.4	1.4	1.4
Uniformity coefficient	2.3	2.3	2.2
Bulk density (kg m^{-3})	365	283	1690
Particle density (kg m^{-3})	1340	1900	2570
Porosity (%)	73	85	34
Specific surface area (m^2 g^{-1})	0.734	> 1000	0.136
Hydraulic conductivity (cm h^{-1})	330	500	360

The specific surface area of the bark was not particularly high, but its chemical composition and surface charge resulted in an active surface. Initially the BOD_5, MBAS, TTC, EHEC and PhiX removal was probably dominated by adsorption mechanisms (**Papers II**). The BOD_5 in the artificial greywater was a result of mixing detergents and cooking oil with nutrient broth, consisting of meat extract and thus containing proteins. Pine bark is rich in tannins (Soto *et al.*, 2005) and studies have shown that bark can effectively bind proteins to tannin (Lewis *et al.*, 1995; Hagerman & Robbins, 1987; Chibata *et al.*, 1986). Lewis *et al.* (1995) also reported remarkable removal of *Enterococci*, *E. coli* and MS2 phages and protein binding in steam-exploded bark.

General biological degradation of organic matter and mineralisation in biofilms (**Papers II** and **IV**; Lens *et al.*, 1993) and degradation of complex substances by fungal flora prevailing on the surface of the bark (Cardoza *et al.*, 2009) are other removal mechanisms. Pine bark is a lignocellulosic material (Miranda *et al.*, 2012), which according to Rowell *et al.* (2012) is porous and may therefore hold water essential to microbial populations harboured by the material. The surface of pine bark is rich in polyhydroxyl and polyphenol functional groups (Bailey *et al.*, 1999), with high cation exchange capacity (CEC) (Naasz *et al.*, 2008; Naasz *et al.*, 2005). Removal of NH_4^+ and Na^+ occurs when the positively charged ions replace H^+ and/or phenolic hydroxyl groups. This results in release of organic acids, which decreased the pH (**Papers II** and **IV**) which is also reported by Genç-Fuhrman *et al.* (2007).

Being lignin-rich, with the major function to protect the tree, bark is usually not easily degraded, particularly when in the form of large fragments. However, constructing the bark filters from small pieces of bark (1- 5 mm with effective size of 1.4 mm) seemed to make the structure vulnerable to degradation. The artificial greywater with its high Tot-N content, as indicated by a TOC:Tot-N ratio of 3.4-4.9 (**Paper IV**), seemed to trigger microbial attacks in scavenging for carbon (**Paper IV**). The weakness of the fine-textured bark was also evident in the water-soluble extractives released as a yellow tint and in the high COD measured in the effluent (**Papers II** and **IV**), which was also reported by Lens *et al.* (1994) when used pine bark for wastewater treatment. Using larger bark particles would probably have decreased the release of the yellow tint, but on the other hand would probably have decreased the specific surface area, leading *e.g.* to less binding of BOD_5 and bacteria. For irrigation purposes, the presence of BOD_5 and pathogens is more serious than dissolved organics forming the yellow tint, which will precipitate on soil minerals after irrigation. Dalva and Moore (1991) reported that soils with low organic matter content and rich in aluminium (AL), iron (Fe) or carbonate are potential sinks for dissolved organics.

The charcoal used as filter material was activated carbon with high specific surface (>1000 m^2 g^{-1}). In order to increase the adsorption capacity of charcoal, after initial pyrolysis it is usually activated by gasification with oxidising gases such as CO_2, steam or air, or by addition of zinc salts or phosphoric acids (Downie *et al.*, 2009). Activation results in increasing porosity and specific surface. In the present case, the newly installed charcoal filters retained 48% of the NaCl used in a hydraulic retention time experiment (**Paper II**) and achieved remarkably high removal of BOD_5, COD, MBAS, Tot-P and Tot-N (Table 3; **Paper II**). Adsorption due to the large specific surface area was the dominant removal mechanism in the initial stages of charcoal filter operation (**Paper II**). In general, the surface of charcoal has hydrophobic sites (consisting of carbon layers) and hydrophilic functional groups (Matsis & Grigoropoulou, 2007), which include OH^-, NH_2, O(C=O)R, CO=OH, C=OH, phenols and carbonyls (Amonette & Josheph, 2009). The different functional groups affect the charge and the acidity of the surface, together with the hydrophobic properties, leading to adsorption of a wide range of different types of pollutants such as NH_4^+ and PO_4, as well as organic matter and bacteria. The charcoal surfaces are finite resources and their adsorption capacity is exhausted by continuous exposure to the pollutants in greywater (**Paper IV**). Nonetheless, the large specific surface area of the charcoal is likely to have hosted large numbers of biofilm bacteria, as well as protecting them from predation by other microorganisms, as suggested by Pietikäinen *et al.* (2000).

The organic matter adsorbed onto the surface of the charcoal provided substrate for the bacteria hosted on the charcoal surface as suggested by Li and DiGiano (1983). This facilitated biological mineralisation of the sorbed organic matter and oxidation of NH_4^+, hence sustaining relatively high BOD_5 and COD reduction and improved nitrification (Table 3; **Paper IV**). Surprisingly, the charcoal did not perform well in removal pathogenic indicators and tracer microorganisms, especially in the later stages of filter life (Table 3; **Paper V**). It can be speculated that even more bacteria would be found in the effluent after clogging of the micropores, because of overgrowth of bacteria on the large specific surface of the charcoal, as discussed by Scholz and Martin (1997).

On comparing the performance of activated and non-activated charcoal for greywater treatment, Berger (2012) showed that non-activated Salix charcoal removed 99% of the COD and MBAS from greywater loaded with 60 g COD m^{-2} day^{-1}, which was similar to the performance of the activated charcoal in the present study. The charcoal studied by Berger (2012) removed Tot-P and PO_4-P more efficiently than activated carbon, with average rates of 89% for Tot-P and 86% for PO_4-P, but the efficiency in removing Tot-N and NH_4^+-N was not stable, whereas activated carbon displayed stable rates of 97% and 98% for Tot-N and NH_4^+-N (Berger, 2012). Another study (unpublished data) comparing activated charcoal with non-activated Salix charcoal at an organic loading of 240 g COD m^{-2} day^{-1} showed no significant difference between the activated and non-activated charcoal regarding COD removal, while the Tot-N removal was significantly higher in the activated charcoal. These results indicate that Salix charcoal could be a good alternative to test in field trials for greywater treatment to produce irrigation water instead of activated charcoal.

The specific surface of the sand was the smallest among the filter materials tested (Table 2), which was probably the reason for the observed low reduction rates of BOD_5 and COD (Table 3; **Papers II** and **IV**). Organic matter degradation by biofilm activity is the dominant removal process in sand (**Papers II** and **IV**) and the rate seemed to be limited by the specific surface area of the sand. Pell and Nyberg (1989a) reported markedly higher organic matter reduction rates in sand filters of 0.21 mm effective particle size, providing a much larger specific surface area.

Table 3. *Mean removal efficiency of greywater pollutants and nitrification in the bark, charcoal and sand filters treating artificial greywater, expressed as a percentage*

Filter medium	Pollutant	New filters 500 mg BOD_5 L^{-1} (**Paper II**)	Increasing HLR 125-500 mg BOD_5 L^{-1} (**Paper IV**)	Increasing OLR 500-2400 mg BOD_5 L^{-1} (**Paper IV** and **V**)
	TSS	92±5	–	–
Bark	BOD_5	98±2	94-96	99±0.1
	COD	74±12	71-40	71-91
	MBAS	>99±0	–	–
	Tot-N	19±9	0-28	7-39
	NH_4^+-N /Tot- N_{ef}	<5	1-2	<1
	NO_3^--N /Tot- N_{ef}	80	68-100	81-94
	Tot-P	97±2	51-59	70-81
	PO_4-P	97±2	51-59	70-82
	TTC	99±1	82-99	–
	EHEC	–	–	96-99
	PhiX	–	–	97-99
Charcoal	TSS	83±8	–	–
	BOD_5	97±3	83-94	94-98
	COD	94±4	76-94	72-88
	MBAS	>99±0	–	–
	Tot-N	98±1	50-83	53-66
	NH_4^+-N /Tot-N_{ef}	5	3-5	1-2
	NO_3^--N /ot- N_{ef}	15	59-67	76-95
	Tot-P	98±1	64-84	86-92
	PO_4-P	98±2	86-93	95-96
	TTC	91±11	65-78	–
	EHEC	–	–	0-82
	PhiX	–	–	51-68
Sand	TSS	56±29	–	–
	BOD_5	75±6	68-85	91-93
	COD	72±2	65-84	66-85
	MBAS	96±1	–	–
	Tot-N	5±7	0-8	0-10
	NH_4^+-N /Tot- N_{ef}	5	2-10	0-3
	NO_3^--N /Tot- N_{ef}	79	57-92	70-100
	Tot-P	83±3	38-62	78-84
	PO_4-P	83±3	27-87	86-92
	TTC	91±11	79-99	–
	EHEC	–	–	40-91
	PhiX	–	–	67-80

Unlike other pollutants, Tot-P removal in the sand filters was relatively high (83-89%) at Tot-P loading rates ranging from 89 to 326 mg m^{-2} day^{-1}. Adsorption is the principal mechanism for PO_4-P reduction in sand filters (Prochaska & Zouboulis, 2003; Pell & Nyberg, 1989b). The capacity of sand to bind PO_4-P depends on pH and the Ca, Fe and Al content in the sand (Arias *et al.*, 2001). Elemental scanning electron microscopy of the sand used in this thesis showed that the particle surfaces contained 22% Ca, 5% Fe and 8% Al (**Paper IV**). Furthermore, the sand contained 2% lime (**Paper II**) and the pH ranged from 6.6-7.7. Hence, PO_4-P binding to Ca hydroxides/oxides was likely to be the prevailing mechanism removing phosphorus in the sand filters.

3.2 Effects of hydraulic properties on pollutant removal capacity

The bark filters had 73% porosity (Table 2). Bark has a high water-holding capacity in relation to sand (**Paper II**) and also swells on exposure to water. The swelling of the bark particles probably resulted in narrowing of the pores, distributing the capillary water more evenly within the filter medium, seen as enhanced removal of TSS (92%) and TTC (92-99%) (Table 3). The effluent from the bark was clear water of yellow tint. More mixing between capillary and non-capillary water might have occurred, leading to increased residence time of the liquid within the pores (43 h) (**Paper II**). The increased contact between bark and water facilitated adsorption and biofilm activities by which BOD_5, NH_4^+-N and PO_4-P were removed (**Paper II**). The long contact time was probably also one factor for the observed high nitrification capacity (Table 3; **Papers II** and **IV**).

The charcoal also had high porosity (85%) (Table 2). Due to the fact that the charcoal was activated, micropores and mesopores (<2 and 2-50 nm, respectively) could be expected to dominate. Matsis and Grigoropoulou (2007) reported that 71% of total pores are micropores in granular activated charcoal. Some grain sizes, between D_{10} and D_{60}, were absent in the charcoal filters, which affected the distribution and size of the macropores and thereby increased the hydraulic conductivity (5.0 m h^{-1}) and shortened the residence time (16 h). This in turn led to a lower reduction in TSS, TTC and BOD_5 in the charcoal filters (Table 3). In micro/mesopores, the dissolved oxygen was probably decreased by bacterial consumption and could even be further aggravated by clogging, leading to anoxic sites. Thus, beside adsorption, loss of nitrogen by denitrification in the anoxic zones could have taken place (Table 3; **Papers II** and **IV**). The micro/mesopores in the charcoal filters seemed to clog by the progressive feeding of the greywater, especially at the high organic loading regime, leading to limited adsorption and hence lower PO_4-P reduction (compared with the initial reduction) (**Paper IV**). Moreover, filtering seemed to decline, resulting in a turbid effluent and very low reduction in EHEC and PhiX phage (Table 3; **Paper V**).

The sand had lower porosity (34%) than the other two filter materials, which could explain the faster flow and shorter residence time (4 h). The fast flow led to inefficient filtration of TSS (56%) and low organic matter reduction (Table 3; **Paper II** and **IV**). Sand filters generally remove TSS by physical filtration and organic matter by filtration and mineralisation in biofilm (Healy *et al.*, 2007; Rodgers *et al.*, 2004). Due to the low porosity in the sand, the capillary water was much lower than in the bark and charcoal filters. Therefore drying of the top surface layer, caused by evaporation under the high ambient temperature of 25-29 °C (**Paper II**), most likely retarded biofilm establishment during the initial stage of the sand filter life, leading to a lower organic matter reduction (Table 3; **Paper II**). Later, when biofilm growth was evident, narrowing the pores (**Paper IV**), the sand filters achieved remarkably high organic matter removal rates (91-93% reduction in BOD_5) (**Paper IV**). Nitrification was efficient in the sand filter (Table 3; **Papers II** and **IV**). Oxygen diffusion to nitrifying bacteria in the sand filter was more pronounced due to the large pores, which limited the denitrification in this filter type compared with the bark and charcoal filters (Table 3; **Papers II** and **IV**).

4 Microbial activity, diversity and composition

Organic matter removal and nitrogen mineralisation in sand filters are accomplished mainly by microbiological activities in biofilms (Leverenz *et al.*, 2009; Healy, 2007; Wanko *et al.*, 2005; Prochaska & Zouboulis, 2003; Pell & Ljunggren, 1996; Pell *et al.*, 1990). The properties of bark and charcoal, as discussed in Chapter 3, suggest that these materials also rely on their biofilm activities when treating greywater. Factors affecting biofilm formation include medium specific surface area, surface charge and roughness (Bolton *et al.*, 2006; Baker, 1984), ability of the surface to absorb organic matter and to adhere microorganisms (Shimp & Pfaender, 1982). In order to understand the full treatment capacity of the three filter materials, exploring their biological activities as well as bacterial community structure (Box 1) is necessary.

The potential respiration activity is useful to give an idea of the total biomass (Anderson & Domsch, 1978) and could possibly also be used to estimate the potential carbon mineralisation capacity of the filters, *i.e.* capacity of the filters to deal with shock loads of organic matter. The potential respiration is usually measured under non-limiting conditions with glucose substrate. Nitrogen mineralisation, *i.e.* conversion of organic nitrogen into NH_4^+, is a general biological process driven by heterotrophic bacteria, while oxidation of NH_4^+ into NO_3^- (nitrification) is mediated by specific lithotrophic bacteria (Tchobanoglous, 2002) and archea (Limpiyakorn *et al.*, 2013; Park *et al.*, 2006). Reduction of nitrite and nitrate to nitrogenous gases, *i.e.* nitrogen removal, is referred to as denitrification (Pell & Wörman, 2008). Nitrification and denitrification in biofilm environments are well documented (Bai *et al.*, 2012; Bassin *et al.*, 2012; Ehlers & Turner, 2012; Almstrand *et al.*, 2011; Chu & Wang, 2011).

Microorganisms involved in wastewater treatment include bacteria, fungi, protozoa, algae (Tchobanoglous, 2002) and archea (Park *et al.*, 2006). In infiltration systems such as sand filters, the microbial ecosystem is dominated by bacteria, but archea, fungi and protozoa may also be important players (Chabaud *et al.*, 2006b; Okubo & Matsumoto, 1983). The microbial ecosystems are dynamic, varying spatially and temporally (Chabaud *et al.*, 2008; Truu *et al.*, 2005), and respond to different substrate and loading conditions (Addison *et al.*, 2011; Pell & Ljunggren, 1996). They are also influenced by environmental stressors and toxic compounds. Therefore, studying the variation in bacterial community structure (Box 1) of the bacteria provides valuable understanding of factors enhancing or suppressing biological activity.

This chapter discusses the effects of greywater application and type of medium on organic matter mineralisation and nitrogen transformation based on results from **Papers II**, **III** and **IV**. The potential of the biofilm developed at OLR of 15 g BOD_5 m^{-2} day^{-1} to treat high glucose was measured using a respiration assay. Filter medium at a depth of 0-2, 20, 40 and 60 cm was sampled in each of the filters every two weeks (**Paper III**). The potential respiration rates of the filter materials were assayed in short-term (24 h) incubations under non-limited organic substrate loadings (2 g glucose) using dissolved oxygen electrode to measure the oxygen consumption. In addition, the effects of greywater and filter medium on bacterial diversity and structure were investigated based on results from **Paper III**. The deoxyribonucleic acid (DNA) of the biofilm was extracted, amplified and sequenced, and bacterial composition was identified. Thereafter, the bacterial community structure (Box 1) was determined.

Box 1. Microbial ecology definitions

- Abundance - Relative number of sequences of a genus to the total number of sequences of genera in the sample.
- Bacterial community - Assemblage of multi types of bacteria living together in a contiguous environment and interacting with each other.
- Bacterial community structure: Bacterial richness and abundance.
- Diversity - Number of OTUs of interest, weighted by some measure of abundance, such as total number OTUs in the community. Often expressed in different indices, e.g. the Shannon index.
- OTU - A group of similar DNA sequences, *e.g.* at 95% similarity level.
- Richness - Number of different OTUs in a community.
- Shannon diversity index - Mathematical measure of diversity in a given community based on the richness and abundance.

4.1 Biological activities

4.1.1 Organic matter mineralisation and potential respiration

Greywater provides a suitable substrate for bacteria in biofilms in terms of carbon and nutrient balance (though shower greywater may be relatively low in nutrients). The amount of organic matter fed to the filters in different operating periods varied within the range 126-2390 mg BOD_5 L^{-1}, meaning that the C:N:P ratio of the artificial greywater varied between approximately 74:14:1 and 150:25:1. According to Chandy and Angles (2001), the C:N:P needed for bacterial growth is 100:10:1, while the actual C:N:P in the microbial biomass itself ranges between 59:12:1 and 100:20:1. Thus, the artificial greywater used in this thesis had a balanced carbon and phosphorus composition in terms of that required for bacterial growth, but excess nitrogen. Greywater is known to be degraded under both aerobic and anaerobic conditions (Abu Ghunmi *et al.*, 2011; Hernández Leal *et al.*, 2011; Zeeman *et al.*, 2008; Hernández Leal *et al.*, 2007), indicating that the organic matter in greywater constitutes an easy available carbon and energy source for bacteria. High degradation capacity was indeed observed for the artificial greywater used in this thesis, even when stored cooled at 2– 4 °C (**Paper II**).

Biological carbon mineralisation is a significant mechanism for organic matter removal in bark (**Papers II-IV**). This was demonstrated by BOD_5 removal rate ranging between 94 and 99% (Figure 2; **Papers II** and **IV**), as well as by the high observed potential respiratory activity of the biofilms (Figure 3). The organic carbon in bark can provide substrate for microbial communities (**Paper II** and **IV**; Trois & Polster, 2007), thus increasing their enzymatic capacity to degrade greywater. This was evident from the high potential respiration rate measured in bark filters fed only with tap water (Figure 3).

Interestingly, the respiration rates were correlated to the number of sequences of subgroups GP1 and GP10 within Acidobacteria bacterial class **(Paper III)**. Naether *et al.* (2012) reported that subgroups belonging to Acidobacteria are common in acidic forest soils and it would not be surprising if conditions similar to those in forest soils (low pH and plenty of complex substrates) prevailed in the bark filters. If the potential respiration rate of the bark fed tap water is subtracted from the rate measured when it is fed with greywater, the difference should in some way represent the increase in microbial respiration potential induced by greywater only.

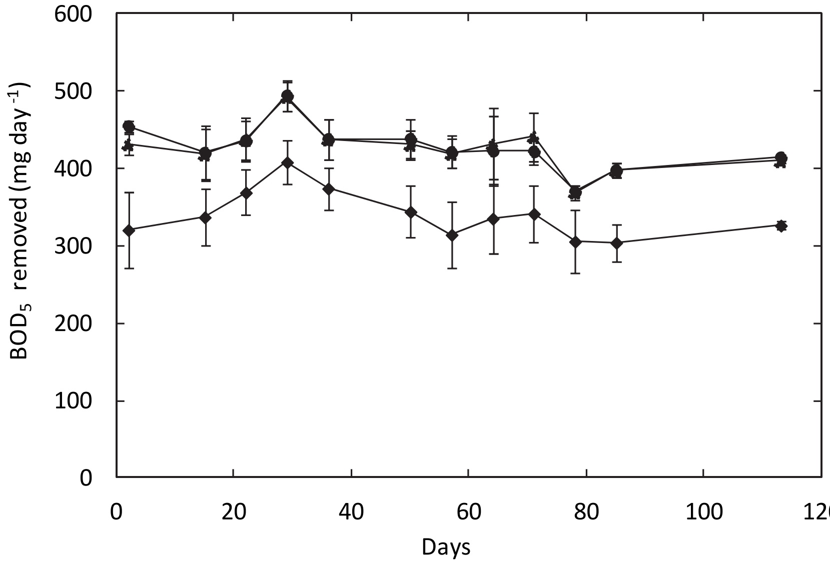

Figure 2. Amount of BOD_5 removed in the bark (●), charcoal (▲) and sand (♦) filters loaded with artificial greywater at 15 g BOD_5 m^{-2} day^{-1} during 116 days (mean ± standard deviation; n = 2) (**Paper III**).

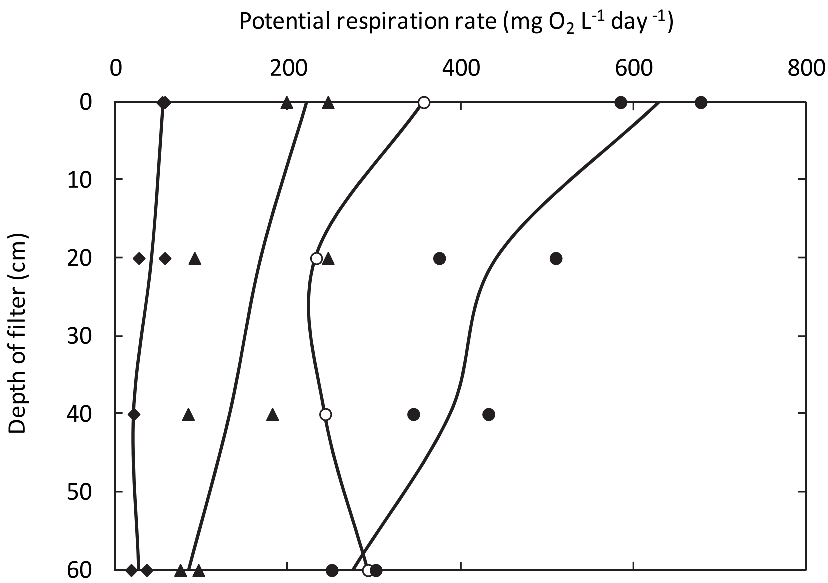

Figure 3. Potential respiration rate (mean, n =2) as a function of depth in bark (●), control bark (○; n =1), charcoal (▲) and sand filters (♦) at day 84 in the infiltration experiment with artificial greywater (**Paper III**).

This difference in potential respiration levels was similar to the potential respiration measured for the more inert charcoal fed with greywater. However, it can be discussed whether the microbes utilised the bark at all, as the greywater substrate was more easily available. One interesting conclusion is that the microbial communities residing on and sustained by the bark can provide extra capacity for efficient removal of organic matter.

Even though the charcoal had similar capacity to the bark to remove organic matter (Figure 2), it displayed lower potential respiration rates (Figure 3). As a consequence of the large adsorptive surface of charcoal, a number of studies have reported creation of a nutrient-enriched environment supporting large bacterial populations (Pietikäinen *et al.*, 2000; Scholz & Martin, 1997; Van Duck, 1984; Shimp & Pfaender, 1982). In contrast, other studies have reported lowered microbial activity induced by charcoal. Thies and Rilling (2009) reported that adding charcoal to soils reduced their respiration. Based on these contrasting observations, it could be speculated that because of the large adsorptive surface of the charcoal, a sparse biofilm might have originally been developed. Further feeding with greywater transformed the charcoal into a biological filter (**Paper IV**), with a more active biofilm. Charcoal has adsorption capacity for different gases including O_2 (Choi *et al.*, 2008). The lower measured potential respiration rates of the charcoal compared with the bark might thus be due to underestimation of the respiration activity. Thies and Rilling (2009) suggested that charcoal may adsorb O_2. Other methods for respiration measurements, such as micro-calorimetric measurements, which depend on measuring heat flow (at micro level) from biological processes (Braissant *et al.*, 2010) or measuring specific enzyme activities such as β-glucosidase (Lazarova & Manem, 1995) might therefore be more suitable than respiration-based methods for measuring the biological activity in charcoal.

The organic matter reduction was less efficient in the sand filters (75%) than in the other filters, and the potential respiration activity rate was also lower than in the other filters (Figure 2 and 3). The ability of the filter surface to adsorb organic matter is important for initial biofilm formation (Shimp & Pfaender, 1982). Newly started sand filters, especially those with small specific surface such as the sand used in the present study (0.136 $m^2\ g^{-1}$), probably do not have significant capacity to absorb organic matter and therefore initially do not support large biofilm cover. *Acidovorax* and *Aquabacterium*, which are reported to be abundant in low biomass biofilms (Liu *et al.*, 2012), were found in high numbers of sequences in both the charcoal and sand filters, indicating low biomass communities, as also suggested by the observed lower respiration rates in these filters compared with bark. Continuous operation of the sand filters, either with high HLR or high OLR, further extended and thickened the biofilm, as shown by its increased capacity to remove BOD_5 (**Paper IV**).

The biofilms in the top layer (0-2 cm) of the bark and charcoal filters had higher respiration capacity than the biofilms at 60 cm depth (Figure 3), while the sand showed similar potential respiration rates in both layers. In the bark and charcoal filters, 34±16% and 63±23%, respectively, of the COD was removed in the 0-2 cm top layer (unpublished data). Well-known biofilm-forming bacteria, such as *Pseudomonas* and *Acinetobacter* (Li *et al.*, 2009), were found in the bark and charcoal filters, with higher abundance at 0-2 cm depth **(Paper III)**. In the sand filters these bacteria were more evenly distributed throughout the sand profile, *i.e.* having similar abundance at 0-2 and 60 cm depth. It is well known that the highest bacterial numbers and activities occur at the top surface (Rajeb *et al.*, 2009; Chabaud *et al.*, 2006a; Pell *et al.*, 1990; Calaway *et al.*, 1952). Therefore, it seems reasonable to argue that most of the organic matter removal occurred in the top 20 cm of the bark and charcoal filters. However, the greywater passed through the sand filter quickly, which decreased the possibility for substrate utilisation by the biofilm **(Paper III).** In a similar context, Bahgat *et al.* (1999) reported no significant difference in numbers of heterotrophic bacteria between the top and bottom of coarse sand filters and attributed this to fast passage of water. At later stages, when the large pores in the sand were bridged with biofilm, the mineralisation of organic matter improved (**Paper IV**). The greywater used in the present study was dosed intermittently onto the filters, which could have regressed the biomass growth and even starved it during times between feedings. This phenomenon particularly influenced the sand filter, as it had the smallest adsorptive surface. Wanko *et al.* (2005) also reported biomass regression and biofilm decay in intermittently dosed sand filters and attributed the biomass regression to oxidation of organics in the absence of substrate and biofilm decay due to dehydration.

4.1.2 Nitrogen transformation and removal

Organic nitrogen represented 16-20%, 31-79% and 21% of Tot-N in the effluent from the bark, charcoal and sand filters, respectively. This indicated substantial nitrogen mineralisation in the bark and sand filters. Adsorption in charcoal is an important process for the removal of different nitrogen forms (organic-N, NH_4^+-N and NO_3^--N), and therefore it can be difficult to assess the mineralisation of nitrogen in this filter material.

The bark, charcoal and sand filters demonstrated effective nitrification, *i.e.* oxidation of ammonium to nitrite and then further to nitrate, but the overall rate was lower in the newly started charcoal filters than in the other filters **(**Table 3;

Papers II-IV). The prevalence of aerobic conditions in all filters, further promoted by the intermittent feeding of greywater (**Paper II**), was probably the reason for the development of successful nitrification. The presence of ammonia-oxidising bacteria (*Nitrosospira* and *Nitrosomonas*) and nitrite-oxidising bacteria (*Nitrospira* and *Nitrobacter*) verified this assumption (**Paper III**). The pH and composition of the medium influenced the composition of ammonia-oxidising genera present in the filters. The relatively acidic environment in the bark filters (pH 5.1) seemed to have retarded the growth of *Nitrosomonas*, which has a reported pH optimum of 7.8-8.5 (Bae *et al.*, 2001; Wild *et al.*, 1971). Instead, *Nitrosospira* dominated and is also reported to exist in acidic and pine forest soils (Koops *et al.*, 2006; Nugroho *et al.*, 2005). Furthermore, the phenolic substances usually found in bark (Miranda *et al.*, 2012) might have been more toxic to *Nitrosomonas* than to *Nitrosospira*. Lauchnor *et al.* (2011) reported phenol inhibition of ammonia oxidation by *Nitrosomonas europaea.* It should be noted that ammonia oxidation may also be accomplished by archeans, as documented for activated sludge (Park *et al.*, 2006) and soil (Leininger *et al.*, 2006), but that was not investigated in this thesis.

From water samples collected at different depths in the bark and charcoal filters, it could be seen that the NH_4^+-N was oxidised to NO_3^--N while infiltrating through the top 20 cm of the columns, indicating high nitrification activity (Figure 4). Similarly, Pell and Nyberg (1989c) reported that under steady state conditions, complete nitrification occurred very rapidly in the top 15 cm layer of sand filter columns fed artificial household wastewater.

The removal of Tot-N was more efficient in the charcoal filter than in the bark and sand filters (cf Figure 4; **Papers II-IV**). Charcoal not only has a higher capacity to adsorb and fix ammonium (Rodrigues *et al*., 2007), but also stimulates denitrification to a higher degree (Zwieten *et al*., 2009). The bacterial genera *Acidovorax*, *Aquabacterium*, *Pseudomonas*, *Bradyrhizobium* and *Rhizobium*, which according to Gómez-Villalba *et al.* (2006), Thomsen *et al*. (2007) and Jones *et al.* (2008) all contain species with denitrifying capacity, were found in all filters. However, it is the environmental conditions that regulate the activity of these genera. Besides nitrate, anaerobic conditions and available carbon regulate the denitrification communities, as reported by Gilbert (2008) and Pell and Wörman (2008). In the charcoal filters, anoxic micropores are likely to have developed and become widespread. This probably stimulated nitrogen loss via denitrification.

Variations in OLR and HLR affected the biofilm activity, which was evident as varying nitrification-denitrification activity (Table 3). Growth of the biofilm in the bark filters in response to increased organic loadings led not only to higher assimilation of nitrogen, but also to improved denitrification. High HLR in the bark and sand filters, on the other hand, decreased nitrification due to shortening the residence time of water and to the development of saturated non-oxic conditions **(Paper IV)**.

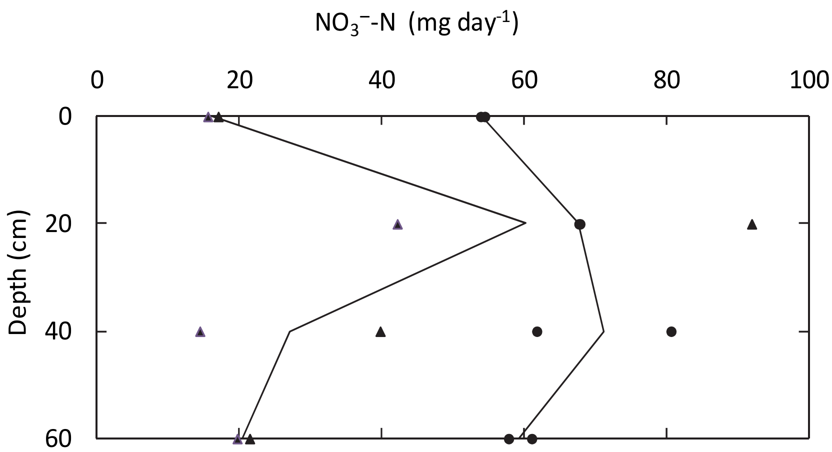

Figure 4. Profiles of NO_3^--N in infiltrated water in bark (●) and charcoal (▲) filters under greywater loading of 13-16 g BOD_5 m^{-2} day^{-1}. Lines represent mean values (n=2).

4.2 Bacterial diversity

In the charcoal and sand filters, more diverse bacterial communities developed, as shown by the scattered patterns in the NMS plot (Figure 5) than for the bark filters, which showed clustered pattern. The artificial greywater was prepared from nutrient broth, cooking oil and detergents and mixed with some real wastewater. These ingredients contain organic macromolecules of a wide range of complexity (carbohydrates, protein and lipids). This is most likely the explanation for more diverse bacterial community observed in the charcoal and sand samples. Though also receiving greywater, the lignocellulosic composition of the bark (Miranda *et al.*, 2012) and the low pH apparently induced a selective pressure on the bacterial community, leading to the development of a more distinct community than in the charcoal and sand materials.

At day 0, before loading with greywater started, the bark had the richest bacterial community (Box 1) expressed as numbers of operational taxonomic units (OTU), 654 compared with 105 OTU in the charcoal and 37 OTU in the sand (**Paper III**). Bark is organic material found in nature and hosts a rich microorganisms when decomposed. Sand and also charcoal to a certain degree are oligotrophic and therefore less favourable for microbial attack. After 14 days of greywater feeding, the microbial richness in the bark, charcoal and sand filters had increased to 686, 540, and 620 OTU, respectively. The bacterial richness (Box 1) of the filters had thus become much more similar. Interestingly, the bacterial diversity (Box 1) and richness of the bark decreased at later stages of operation **(Paper III)**. Greywater application to the bark filters seemed to disadvantage some bacterial genera, as was apparent from the successive disappearance of *Kofleria, Methylocystis*, *Acidocella* and *Herbaspirillum* in the bark filters.

In the bark and charcoal filters, richer communities were observed after 14 days at 0-2 and 20 cm depth compared with that at 60 cm, indicating an active treatment zone in bark and charcoal of less than 60 cm. This agreed with the results from the potential respiration rate measurements and nitrate profiles (Figures 3 and 4). In the sand filters, rich community also developed at 60 cm due to that more untreated material had made its way to 60 cm depth than in the bark and charcoal filters. This, together with the water-logging at deeper levels of the sand filter, enriched the bacterial communities in the sand and allowed the development of rich biofilm even at the bottom of the column. It would have been interesting to see how the bacterial diversity and composition at 40 cm and to see if this community is related to that of 0-2 or 60 cm depth. Such information could probably assist in evaluating the extent of the active infiltration zone and the actual column depth needed for complete purification.

The bacterial diversity in the three filter materials displayed lower Shannon diversity index values (1.75-2.3) than those reported for different soil ecosystems (5.39-7.07) (Chau *et al.*, 2011; Kim *et al.*, 2006; Dunbar *et al.*, 2000). The more complex and environmentally diverse ecosystem in soil is probably the reason for its higher bacterial diversity. Perhaps more surprisingly, the bark, charcoal and sand filters showed lower bacterial diversity than activated sludge, which has a reported Shannon index of 6.90-7.36 (Hu *et al.*, 2012). The activated sludge process varies between aerobic, anoxic and anaerobic conditions, and has a large inflow rich in different types of bacteria coming from blackwater, *etc*. The greywater composition and the operating and environmental conditions in the present study were fairly constant, which defined the bacterial community structure with the lower diversity compared with that in soil and activated sludge.

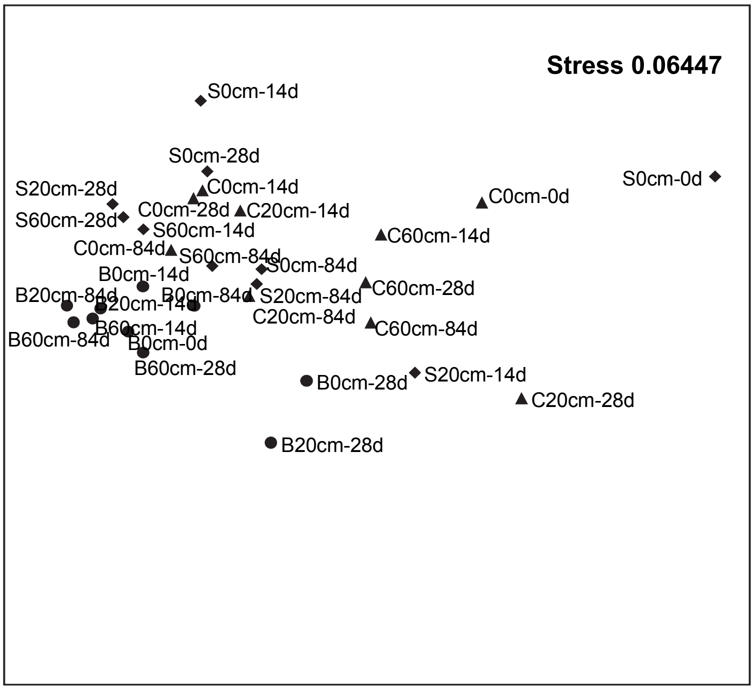

Figure 5. Non-metric multidimensional scaling (NMS) plot showing similarities in bacterial composition at class level between bark (●), charcoal (▲) and sand (♦) filters at the indicated horizon and incubation time (Paper III). Points close to each other have a similar bacterial composition.

5 Loading conditions and treatment capacity

Greywater flows vary daily, weekly and seasonally, which affects the treatment processes (Eriksson *et al.*, 2009; Ghunmi *et al.*, 2008). The HLR affects flow velocity, oxygen transportation and degree of water saturation in the pores (Rolland *et al.*, 2009; Boller *et al.*, 1993), thereby regulating the oxygen status of the filter material and its oxidation of pollutants. Also, on the one hand, high HLR increases the wetted area in granular filters with unsaturated flow (Sharvelle *et al.*, 2008), which in turn increases the effective infiltration surface and thereby improves the conditions for biofilm coverage. On the other hand, high HLR imposes shear stress on the biofilm surface due to the higher water speed (Trulear & Characklis, 1982). Peak loading conditions can also lead to temporary failure of the treatment system, but on the other hand, the effective filtration area/depth might not be utilised at low hydraulic loads, which is economically inefficient.

Greywater composition also varies with time (Jefferson *et al.*, 2000). When the flux of organic matter to the biofilm increases, the biological activity of the microorganisms is stimulated (Trulear & Characklis, 1982), leading to higher mineralisation rates of organic matter (Wijeyekoon *et al*., 2004). Intensive organic loading leads to dense biofilm formation, whereupon flux of substrate and oxygen into the interior of the biofilm is limited (Wijeyekoon *et al*., 2004). Ultimately, this will result in biofilm detachment, which can lead to emission of pollutants (Li *et al.*, 2012).

Loading frequency and resting periods also influence the performance of the filters. Intermittent dosing can open filter pores during periods of drought, allowing oxygen diffusion into the filters (Stefanakis & Tsihrintzis, 2012), which enhances the mineralisation of stored organic matter. This expands the service life of well-functioning filters and may restore overloaded filters (EPA, 2002; Pell, 1991).

Knowledge about the response of filter media to loading and operating conditions is decisive in determination of their treatment capacity so as to avoid overloading or failure events. In the following sections, the response of bark, charcoal and sand filters to variable hydraulic and organic loading rates in terms of BOD_5, COD, Tot-P, Tot-N and tracer organisms reductions is discussed. The effects of varying HLR and OLR were tested in a series of experiments using the column set-up described in Chapter 1 (**Paper II**). HLR was studied by increasing it stepwise from 32 to 128 L m^{-2} day^{-1} and then restoring it again to the initial 32 L m^{-2} day^{-1} while keeping the OLR constant at 13-16 g BOD_5 m^{-2} day^{-1}. OLR was studied by increasing it from 14 to 76 g BOD_5 m^{-2} day^{-1} and then decreasing it to 13 g BOD_5 m^{-2} day^{-1} while keeping the HLR constant at 32 L m^{-2} day^{-1} (Figure 6A). Each loading regime was applied for three weeks until an assumed steady state was reached. The effects of HLR and OLR on the reduction in pollutants and on nitrogen and phosphorus mineralisation were modelled using general regression models (GRM; Table 4).

5.1 Hydraulic loading rate

The different materials responded differently to the HLR (HLR_i and HLR_{i-1}; Table 4). In the bark filters, for instance, increasing the HLR from 32 to 128 L m^{-2} day^{-1} successively decreased the capacity to remove BOD_5 (from 95 to 93%), COD (from 74 to 40%), Tot-P (from 62 to 56%), NH_4^+-N (from 97 to 88%) and also decreased the NO_3^--N fraction (from 1 to 0.64) and TTC reduction (from 96 to 82%) (**Paper IV**). The instant effect of increasing the HLR was an increase in flow velocity in the filters, as evident from the shortest residence time (Figure 6B). Shortest residence time is the time lapse between the greywater dosage and the first outflow from the filters, repeatedly determined throughout the experiment (**Paper IV**). The faster flow rates shortened the contact time of BOD_5, Tot-P and NH_4^+-N with the filter medium and its biofilm and thereby caused some pollutant fraction to pass through without being treated (**Paper IV**), as also suggested by Li *et al.* (2012). Therefore, when HLR was decreased, the reduction in pollutants increased again (**Paper IV**). Moreover, under high flow rates biofilm detachment due to fluid shear stress is likely to occur (Trulear & Characklis, 1982). Part of the biofilm in the bark seemed to be shed, which increased the pollutants slightly in the effluent (**Paper IV**). The chemical and physical composition of the bark material interfered with the HLR, particularly regarding the COD reduction. Pine bark contains water-soluble compounds (Miranda *et al.*, 2012), and when more water percolated through the filters, more soluble compounds were washed out from the bark (**Paper IV**). This increased the COD content of the effluent, resulting in an apparent lower capacity of the filter to treat the greywater.

Increasing the HLR of the charcoal and sand filters from 32 to 128 L m^{-2} day^{-1} increased their capacity to remove organic matter. At increasing load, the BOD_5 reduction improved from 87 to 92% in the charcoal filters and from 76 to 84% in the sand filters, while the removal rate of COD increased from 76 to 90% and from 67 to 83%, respectively. Moreover, at increasing HLR the NH_4 reduction decreased from 95 to 88% in the charcoal filters but increased from 79 to 95% in the sand filters. The TTC reduction in the charcoal filters ranged within 65-78% with no significant difference between the different HLR regimes, but it decreased from 92 to 79% in the sand filters. Increasing the HLR increased the wetted area, which is also suggested by Sharvelle *et al.* (2008) and Siegrist *et al.* (2000), which improved the conditions for biofilm coverage and for adsorption (**Paper IV**). Thus the active purification surface and depth increased, explaining the higher organic matter removal by microbial degradation in the charcoal and sand filters and the more efficient adsorption in charcoal (**Paper IV**). Aijiao *et al.* (2008) showed that the performance in terms of COD reduction in charcoal filters improves as flow rate increases. The question then is why the increased flow velocity associated with increasing HLR lowered the NH_4^+-N reduction, but not the BOD_5 reduction. It is possible that the biological activity responsible for organic matter degradation was faster than that governing oxidation or adsorption of NH_4^+-N. Hence, NH_4^+-N was more sensitive to higher HLR within the span of the investigation. Zou *et al.* (2009) reported a drop in NH_4^+-N removal as hydraulic loading increased and explained this by the shortened residence time.

Interestingly, the GRM statistics proved that the pollutant reduction in the filters was influenced not only by the hydraulic loading applied at present run (HLR_i), but also that at the previous run (HLR_{i-1}). The loads applied as far back as three weeks before the measurements (HLR_{i-1}) had a clear impact on filter performance, *i.e.* BOD_5 reduction, nitrogen transformation and phosphorus reduction (Table 4; **Paper IV**). A number of physical, chemical and biological processes in a porous medium govern the organic matter, nitrogen and phosphorus transformations and bacterial growth. These processes are influenced by residence time, flow velocity, pH and amounts of oxygen. HLR has been demonstrated to affect the tracer (bromide) residence time (Van Cuyk *et al.*, 2001), water saturation conditions and oxygen transportation (Rolland *et al.*, 2009; Boller *et al.*, 1993), as well as flow velocity. Therefore, changes in HLR in the present experiment would have altered these parameters, which in turn influenced the fate of pollutants and treatment efficiency even in the following run.

Table 4. *Summary of GRM coefficients (a-e), coefficient of determination (R^2) and probability value (p) of models for the pollutant reduction in bark, charcoal and sand filters subjected to varying hydraulic (HLR_{i-1} and HLR_i) and organic (OLR_{i-1} and OLR_i) loading rates of artificial greywater*

Filter	Pollutant	Intercept a	HLR_{i-1} b	HLR_i c	OLR_{i-1} d	OLR_i e	R^2	p
Bark	BOD_5	0.9516	0.0004	-0.0004	-0.0007	0.0007	0.61	0.000
	COD	0.8144	-	-0.0036	-	0.0023	0.82	0.000
	Tot-P	0.9093	0.0021	-0.0021	-0.0180	0.0066	0.98	0.000
	Tot-N	0.03956	-	-	-0.0060	0.0065	0.75	0.000
	NH_4^+-N	0.9970	-	-0.0009	-	0.0002	-	0.000
	NO_3^--N /Tot-N	1.2813	-0.0062	-0.0038	-	-	0.48	0.000
	NH_4^+-N /Tot-N	0.0116	-	-	-	-0.0002	0.37	0.000
Charcoal	BOD_5	0.8438	0.001	0.0001	-0.0012	0.0012	0.61	0.000
	COD	0.7168	-	0.0014	-	-	0.64	0.000
	Tot-N	0.8500	-	-0.0024	-0.0084	0.0084	0.61	0.000
	NH_4^+-N	0.9841	-0.0008	-	-	-	-	0.000
	NO_3^--N /Tot-N	0.4609	0.0023	-0.0023	-0.0084	0.0013	0.74	0.000
	NH_4^+-N /Tot-N	0.0579	-0.0002	0.0002	-0.0005	0.0005	0.43	0.000
Sand	BOD_5	0.6491	0.0021	0.0001	-0.0018	0.0044	0.53	0.000
	COD	0.5744	-	0.0016	-	0.0032	0.54	0.000
	Tot-P	0.6208	0.0023	-0.0023	-0.0065	0.0065	0.83	0.000
	NH_4^+-N	0.6836	0.0015	0.0003	0.0019	0.0019	0.45	0.000
	NH_4^+-N /Tot-N	0.1036	-0.0004	-0.0003	-0.0007	-0.0005	0.34	0.003

- Coefficient value not within the 95% confidence interval and therefore omitted.

5.2 Organic loading rate

The bark, charcoal and sand filters showed similar responses to increasing OLR (OLR_i and OLR_{i-1}) when it was increased from 14 to 76 g BOD_5 m^{-2} day^{-1} (Table 4). With few exceptions, the BOD_5, COD, Tot-P and Tot-N, PhiX and EHEC reductions increased with higher loading, from 99 to 100%, 73 to 87%, 81 to 73%, 0 to 29%, 97 to 99.9% and 96 to 99% in the bark filters, from 93 to 98%, 76 %, 83 to 98%, 66 to 63%, 52 to 67% and 82 to 0% in the charcoal filters and from 93 to 99%, 70 to 86%, 83 to 93%, 4%, 68 to 80% and 90 to 40% in the sand filters. In addition, the quantity of pollutants removed increased, even when the reduction percentage decreased. The effects of organic loading on the reduction in COD and Tot-N in charcoal and Tot-N in the sand were not statistically significant. When the OLR increases, the substrate flux to the biofilm increases, which stimulates the activity of the microorganisms (Wilson *et al.*, 2011), leading to higher mineralisation rates of organic matter (Wijeyekoon *et al.*, 2004). Under high loadings with organic matter, thicker biofilm seemed to grow in the bark and sand filters, which agreed with findings reported by Bassin *et al.* (2012) and Tijhuis *et al.* (1994). Thicker biofilm increased the resistance to the greywater flowing through the filters, which in turn prolonged the water residence time (Figure 6B; **Paper IV**) and allowed more efficient organic matter and PhiX removal, particularly in the bark and sand filters (**Papers IV** and **V**). The prolonged observed residence time indicated a decrease in hydraulic conductivity in the bark and sand filters with increasing OLR. Formation of biofilm has been reported to decrease the hydraulic conductivity in filters treating wastewater (Beal *et al.*, 2006; Beach *et al.*, 2005; Siegrist & Boyle, 1987). Such effects could have happened in the charcoal filters, but were not observed, perhaps because of the high initial hydraulic conductivity obscuring small changes (Chapter 3). Besides adsorption of Tot-P to the filter materials, microbial assimilation of phosphate in the growing biofilm is inevitable (**Paper IV**). However, in the bark filter, anoxic incidences probably occurred, which led to desorption of PO_4-P (**Paper IV**). Fluctuating oxygen conditions also favoured denitrification, which increased in the bark filters, further supporting the occurrence of anoxic zones. The removal of Tot-N in the charcoal decreased with decreasing OLR, and at the same time the nitrification increased. Yet, the charcoal filters still achieved the highest Tot-N reduction (63-66%) compared with both the bark (0-29%) and sand filters (4%) (**Paper IV**).

The GRM statistics showed that filter performance was influenced by the organic loading rate in the present run (OLR_i) as well as the organic loading in the previous run (OLR_{i-1}). This effect on filter performance was particularly evident when the high OLR was suddenly decreased (**Paper IV**). At that time, the previously flourishing bacterial community suddenly experienced starvation followed by biofilm detachment, which led to nitrogen and phosphorus assimilated in the biofilm during the preceding run being mineralised and leaking out (Table 4; **Paper IV**). In a similar context, Ahn *et al.* (2006) studying microbial adaptability in biological phosphorus removal systems, reported release of phosphorus after an abrupt decrease in OLR, which they explained by the severe upset of the bacterial metabolism in the system. It could be speculated that the effects from the previous loading are not long-lasting and thus when excess biomass has been mineralised and the biofilm is in balance again, the reduction capacity will increase.

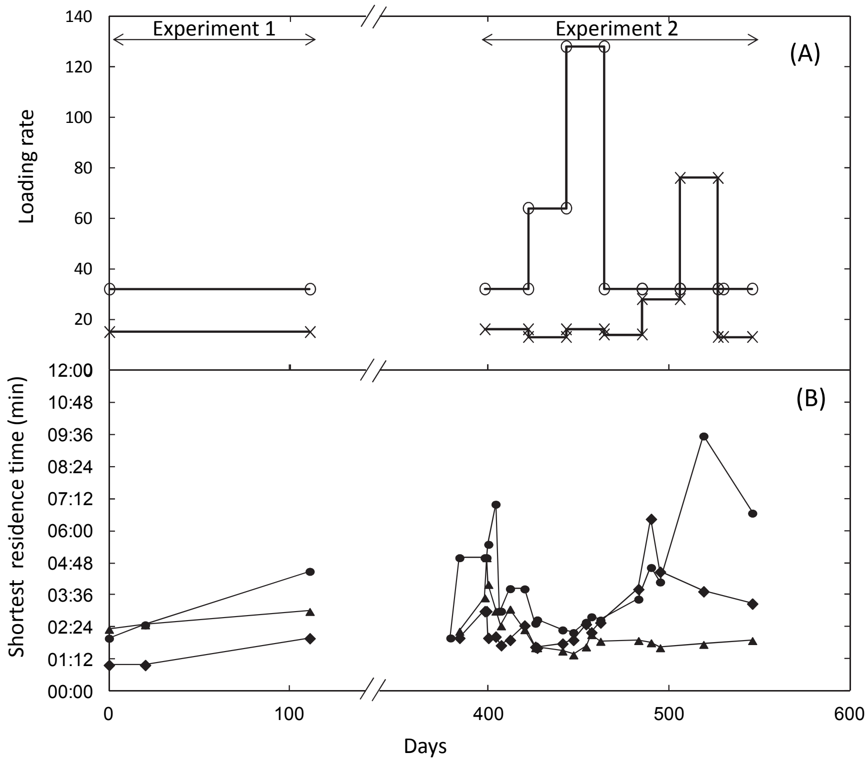

Figure 6. A) Hydraulic (L m^{-2} day^{-1}) (○) and organic loading rates (g BOD_5 m^{-2} day^{-1}) (×) in the first (**Paper II**) and second (**Paper IV**) experiment. The truncation represents the periods when the filters were resting. B) Shortest residence time in the bark (●), charcoal (▲) and sand filters (♦) during the different loading regimes.

5.3 Intermittent loading

Greywater generation is usually intermittent and therefore most on-site treatment systems are fed intermittently. To mimic this, the filters in the present study were dosed intermittently with three doses per day (**Paper II-V**), with the longest resting time between the doses being 13 hours. Intermittent dosing can generally be predicted to enhance organic matter degradation and nitrification in the filters. Resting times allow oxygen to diffuse into the filter (Stefanakis & Tsihrintzis, 2012), hence allowing bacteria to mineralise organic matter stored within the pores and to nitrify ammonium. Besides, intermittent loading controls the growth of biomass, decreases the risk of clogging and hence extends the service life of the filter (Leverenz *et al.*, 2009).

Long resting periods, such as the eight months which elapsed between the studies in this thesis, had the same effects as discussed above plus nearly complete biofilm decay, as also suggested by Pell (1991) and Bancolé *et al.* (2004). This indicates possibilities for reliable rehabilitation/restoration of filters for renewed start-up. The long resting period also led to mineralisation of biologically assimilated nitrogen and phosphorus, producing ions. Therefore, PO_4-P and NO_3^--N were washed out with water before the filters were used in the next experiment. During the long resting period significant amounts of water evaporated from the filter pores. This was particularly important for the bark filters, where the chips swell upon wetting and then shrink again on drying. The swelling-drying caused disruption of the bark chips and therefore at the second start-up some small particulate material was moved through the filter, causing fluctuations in the observed shortest residence time (Figure 6).

5.4 Treatment capacity of the filters

The BOD_5 removal capacity of the filters during the OLR experiment (13-76 g BOD_5 m^{-2} day^{-1}) was evaluated in **Papers II** and **IV**. In addition, the potential treatment capacity (PTC) of the filters was calculated using the potential respiration rates measured in **Paper III** and further discussed in Chapter 4. The total amount of oxygen respired in the entire filter was calculated by measuring the potential respiration rates at four depths of the filters and then integrating the potentials along the filter length.

Within the 13-76 g BOD_5 m^{-2} day^{-1} loading range, the bark filters achieved 94-99% removal efficiency, which was the highest treatment efficiency of organic matter among the three filter types (Table 5; **Papers II** and **IV**). The bark filters demonstrated PTCs of 3.4 and 17.5 times the maximum and minimum loads applied in the OLR experiment (Table 5). In a full-scale application, Lightsey *et al*. (1976) reported a 95% reduction in BOD_5 in pine bark trickling filters loaded with 55 g BOD_5 m^{-2} day^{-1}. Based on the calculated PTC of the bark filters, it could be speculated that bark filters have the capacity to accept and remove short-term shock loads of BOD_5. However, the PTC is only indicative and cannot be used to draw conclusions on the long-term treatment capacity of the filters, as it does not consider biomass growth or accumulation in the filters. At the highest OLR applied in the experiment (76 g BOD_5 m^{-2} day^{-1}), a slowdown in the hydraulic flow in the bark filter was observed (Figure 6), which could be a first sign of emerging clogging. However, operating the bark filters until failure, *e.g.* water ponding at the surface, is needed to determine the real long-term treatment capacity of these filters.

Table 5. *Potential treatment capacity (PTC) of bark, charcoal and sand filters in terms of BOD_5 removal under different measured or calculated loading rates*

Loading conditions	Applied BOD_5 ($g\ BOD_5\ m^{-2}\ day^{-1}$)	Removed BOD_5 ($g\ BOD_5\ m^{-2}\ day^{-1}$)		
		Bark	Charcoal	Sand
Minimum loading	14	14±1.5	13±1.7	12±1.9
Maximum loading	76	76±0.2	72±1.0	71±0.3
Potential treatment capacity	>2000	262±55	83±35	33± 38

The measured BOD_5 treatment capacity of the charcoal filters was lower than that of the bark filters. The charcoal filters removed 92-95% of the applied load (Table 5; **Paper IV**). The calculated PTC was 5.7 times the minimum applied load in the OLR experiment, but was within the range of the maximum applied load (Table 5). The calculated PTC only considers the biological potential, without taking into account the adsorption capacity of the filter material. Upon depletion of the adsorption capacity, *i.e.* after a certain period of operation, the charcoal will start acting as a genuine biological filter. Generally, the BOD_5 treatment capacity of the charcoal filters was lower than that of the bark filters, despite the charcoal having a much larger specific surface (Table 2). The underestimation of the respiration potential, discussed in section 4.1.1, was reflected on the calculated PTC. Furthermore, the contributing activity of anaerobic bacteria residing in the charcoal pores was not considered when calculating the PTC by use of an oxygen respiration method. Considering the non-accounted capacities of the charcoal, *i.e.* adsorption and anaerobic activity, the treatment capacity of the charcoal filters could be expected to be higher than the estimated PTC.

At later stages of operation of the sand filters, the treatment capacity increased and ranged within 85-93% of the applied loads. The calculated PTC was only twice the minimum load in the OLR experiment, and the maximum treatment capacity was within the variations of the PTC (Table 5). Based on carbon dioxide release, Pell *et al.* (1990) estimated a potential treatment capacity of 89-134 g BOD_5 m^{-2} day^{-1} for fine sand (D_{10} of 0.21 mm). The recommended design factor for OLR of coarse sand filters is about 5 g BOD_5 m^{-2} day^{-1} according to EPA (2002). These design loads must be considered very low compared with the actual loads applied in this thesis. However, such organic design loads are most likely intended to minimise the long-term risk of failure by clogging.

5.5 Service life of the filters

The service life of a filter can be defined as the time span during which the filter is expected to deliver acceptable removal rates of various pollutants. Service life is particularly important to consider for bark filters, because their organic origins make them more susceptible to degradation than the inert materials of charcoal and sand. Several studies have documented the fate of bark during composting (Davis *et al*., 1992; Trois & Polster, 2007) and similar conditions to those in the composting process might prevail in bark filters, leading to gradual degradation of the material and thus decreasing its service life. The greywater used in the present study was rich in nitrogen and phosphorus (**Papers II** and **IV**) and also contained a rich mixture of microbes (**Paper III**). Attacks on the bark by heterotrophic microbes to obtain carbon could have occurred (**Papers II-IV**). High respiration rates were observed in samples collected from the bark filters fed with tap water, which indicated activity of some microorganisms degrading the bark material (**Paper III**). Moreover, the swelling-shrinking cycles of the bark due to periods of wetting and drying could be expected to weaken the structures, causing disintegration of the bark particles. This would lead not only to loss of filter medium but also clogging phenomena. Applying greywater of high organic strength to bark filters was suggested to protect the bark from microbial attack due to both the biofilm coating physically protecting the material and to preferential consumption of the more easily available carbon fed with the water (**Paper IV**). However, the relationships between the organic load and rate of degradation and the service life of bark filters need more research. Charcoal and sand are oligotrophic and therefore biodegradation of the medium itself is not a concern. The service life of charcoal also needs more research regarding its adsorption capacity of organic material and subsequent biological degradation of the sorbed matter. Design of sand filters for long service life (*e.g.* 30 years) has been well studied (EPA, 2002; Tchobanoglous, 2002; Joseph A *et al.*, 1969; McGauhey & Winneberger, 1964).

6 Recycling opportunities and constraints

Agriculture is the sector using most water, 70% of total global water use (FAO, 2007). Therefore reuse of treated greywater should be seen as an important contributor to sustainable water conservation.

The ultimate goal of this thesis was to develop filters for producing treated greywater suitable for irrigation. In this chapter, the opportunities and constraints in recycling greywater for irrigation are discussed. The discussion is mainly based on greywater quality in rural communities in Jordan (Suleiman *et al.*, 2010; Halalsheh, 2008). The greywater was assumed to be treated in bark, charcoal or sand filters, after which the removal efficiency of organic matter, nitrogen, phosphorus and microbial indicators found in the filter experiments in **Papers II-V** were assumed in the following discussion. Furthermore, the suitability of the effluent for irrigation was assessed based on Jordanian standards for use of treated wastewater. The assessment was limited to nutrients, organic matter and hygiene quality properties and hence salinity issues and metal concentrations were not considered.

6.1 Water for irrigation

Greywater should be seen as a sustainable water resource to be used for irrigation. In Jordan, where the per capita water share is less than 200 m^3 per year (FAO, 2007), saving every drop of water is a necessity. In rural areas of north-east Jordan, separation of greywater from toilet blackwater is already practised (Suleiman *et al.*, 2010). The per capita daily greywater generation in north-east Jordan ranges from 83-140 L day^{-1} (Dalahmeh, 2010; Halalsheh, 2008). The incentive for recycling treated greywater for irrigation in the rural setting is strengthened by land availability close to the household. Olives are a common crop in Jordan, and can be irrigated with treated greywater to good effect. With drip irrigation or localised irrigation, the annual water requirement for olives in Jordan is 400 L m^{-2} $year^{-1}$ (El Zuraiq *et al.*, 2004). Thus, 74-124 m^2 of land could be irrigated with greywater generated in a typical household in the rural community, which agrees well with the normal garden area. Jamrah and Ayyash (2008) reported that garden irrigation accounts for 5-7% of total water consumption in major cities of Jordan, which implies that water savings of this magnitude should also be realistic for rural areas. Mandal *et al.* (2011) reported a 48% saving in water consumption when greywater was collected, treated and recycled for irrigation in one community in India, while Mourad *et al.* (2011) reported a 35% water saving by recycling treated greywater for toilet flushing.

6.2 Organic matter

Treating rural household greywater in a bark or charcoal filter, assuming treatment capacities obtained in **Papers II-V**, should substantially decrease the level of easily degraded organic materials in the greywater (Table 6), which would solve the problem of odours when the water is stored before use. The remaining organic material in the treated water can be considered a surplus contributing to soil organic matter content and sustaining soil microbial activity when irrigated to the soil system. Jordanian soils are usually poor in organic matter, with contents normally ranging between 0.07 and 0.9%. The bark effluent used in **Papers II** and **IV** contained total dissolved organic carbon (TOC) also originating from the bark itself (20 mg TOC L^{-1} from bark fed with tap water and 80 mg TOC L^{-1} from bark fed with greywater). The dissolved organic carbon (DOC) might be precipitated by soil minerals, hence adding organic matter content to the soil. Dalva and Moore (1991) reported that soils low in organic matter but rich in Al, Fe or $CaCO_3$ are potential sinks for DOC. The agricultural soils in Jordan are generally alkaline (pH 7.9-8.4) and contain $CaCO_3$ (8-25%) (El Zuraiq *et al.*, 2004). However, care should be taken not to irrigate soils contaminated with heavy metals, because DOC might increase the mobility of metals such as copper, chromium and lead, which are of special concern at low soil pH (Fest *et al.*, 2008; Linde *et al.*, 2007; Wu *et al.*, 2002). Depending on the quality of the treated wastewater, the Jordanian standards on treated wastewater for use in irrigation allow three classes of crops to be irrigated (Table 6). Comparing the calculated greywater quality with the Jordanian standards, BOD_5 in the bark effluent met the standards for all classes, while charcoal and sand met the BOD_5 requirements for classes two and three (Table 6). The COD in the effluent from all filters did not meet the standards for any of the classes.

Table 6. *Quality of real greywater generated in rural settings in Jordan, and calculated quality of the greywater assuming the treatment efficiencies in bark and charcoal filters found in this thesis. Classes 1-3 are the Jordanian standards for use of treated wastewater in irrigation*

					Jordanian standards for use of treated wastewater in irrigation		
					Class 1	Class 2	Class 3
	Greywater from rural setting in Jordan	Bark effluent [a]	Charcoal effluent [a]	Sand effluent [a]	Cooked vegetables [e]	Fruit trees [e]	Field crops, industrial crops and trees [e]
pH	64 [b]				6.5-9	6.5-9	6.5-9
TSS (mg L^{-1})	845[b]	8-25	270-346	169-279	50	150	150
BOD_5 (mg L^{-1})	1060[b]	10-11	21-64	74-95	30	200	300
COD (mg L^{-1})	2570[b]	231-745	308-720	386-874	100	500	500
Tot-N (mg L^{-1})	211 [c]	129-196	72-99	190-211	45	70	70
Tot-P (mg L^{-1})	20[b]	4-6	2-3	2-4	30	30	30
E.coli (MPN $100mL^{-1}$)	2×10^5 [b]	2×10^3 -3.6×10^4 [d]	4.4×10^4 - 7×10^4 [d]	2×10^3 – 4.2×10^4 [d]	100	1000	

[a] Calculations based on the treatment efficiencies obtained for bark and charcoal and sand for BOD_5 concentrations in the influent of 500-2000 mg L^{-1} and presented in Table 3.

[b] Halalsheh (2008)

[c] Suleiman *et al*. (2010)

[d] *E. coli* not analysed in this work, so removal assumed to be similar to that achieved for *TTC*

[e] JISM (2006)

6.3 Nutrient availability

The need for plant nutrients in cultivation depends on the crop. Assuming that olive trees are irrigated with the treated greywater and based on the effluent content of mineral nitrogen and phosphorus reported in **Papers II-IV**, irrigation of a 74 m^2 garden with 400 L m^{-2} $year^{-1}$ (irrigation requirement for olives) of greywater treated in bark, charcoal or sand filters would result in the application of 430-658 kg N ha^{-1} and 12-21 kg P ha^{-1}; 227-313 kg N ha^{-1} and 2.6-4.5 kg P ha^{-1}; and 630-700 kg N ha^{-1} and 3.4-11 kg P ha^{-1}, respectively. In olive production, the mineral N and P recommendation for traditional olive cultivation in the Mediterranean countries is 75-150 kg N ha^{-1} (0.5-1 kg N per tree) and 4.5-6 kg P ha^{-1} (30-40 g P per tree) (Ibrahim *et al.*, 2009). In view of the irrigation requirement of olives and the fertiliser value of the effluent from all filter types, the nitrogen requirement of the olive crop would be fulfilled with a large surplus. The positive side of having nutrients in the effluent is that the need for other fertilisers is reduced, thereby mitigating the costs for purchasing mineral fertiliser and also the demand for energy inputs during its application. However, the excess nitrogen may pose a risk of pollution if the surplus water runs off to water courses or infiltrates to the groundwater (**Paper II**), which means noncompliance with the Jordanian standards (Table 6). The nitrification capacity in the filters is high and hence the effluent is rich in NO_3^- -N, which will increase the mobility of the nitrogen in the soil system and thus the risk of it contaminating drinking water resources. With well-managed, water-saving irrigation systems, such as drip irrigation, the risk of nitrogen leaching to groundwater should be low and the nitrogen can therefore be considered a pure asset. In arid regions, alternating irrigation with fresh water might be needed to 'dilute' the nutrient concentrations and thus avoid excessive application. Irrigation water is normally scarce and therefore not applied in larger amounts than necessary to compensate for evapotranspiration. Dilution, *i.e.* using greywater as a supplement to another source of water without plant nutrients, has the advantage that the plant nutrients can be spread over a much larger area, thus replacing and saving much more chemical fertiliser. If there is such an alternative source of water, the greywater would be distributed over a larger area than stated above, which would supply just sufficient amounts of nitrogen, 75 kg N ha^{-1}.

The need for phosphorus fertilisation depends on how much phosphorus is stored in the soil (Jordan-Meille *et al.*, 2012) and how available it is. The phosphorus content ranges from 4.9-14 mg Tot-P kg $soil^{-1}$ in soils in Jordan. Excess phosphorus might not be a problem, as it can be adsorbed to the soil. However, the effluent from the charcoal filter might be deficient in mineral phosphorus. It should be pointed out here that greywater quality varies and greywater from some settings might be low in nutrients. After treatment, such greywater can be considered just a water source for irrigation, rather than a fertiliser.

6.4 Hygienic quality

The TTC analyses in this thesis indicated reductions in this bacterial indicator of 1-2 $\log_{10}$ in the bark, charcoal and sand filters, with bark being slightly more efficient. The charcoal and sand effluents contained about 2 $\log_{10}$ of *Enterococcus* spp. as a result of presumed growth within the filters (**Paper II**), which might pose a risk upon recycling. Lewis *et al.* (1995) reported 3 $\log_{10}$ removal of *E. coli* and *Enterococcus fecalis* in bark of *Pinus radiata* treated by steam-explosion. In **Paper V**, the bark filters showed the highest removal rates of EHEC (1-3 $\log_{10}$) and the phage PhiX (2-3.7 $\log_{10}$), while charcoal showed the least capacity to reduce these tracer microorganisms (0-0.7 $\log_{10}$ for EHEC and 0.3-0.5 $\log_{10}$ for PhiX) among the three filter types. The high reduction of PhiX by the bark suggests that some human viruses might also be removed by this filter material. However, the effectiveness of virus removal might depend on their size, surface charge and other characteristics such as presence of an envelope.

The Jordanian standards regarding treated wastewater for irrigation use *E. coli* as an indicator to assess the microbial quality of the wastewater. As *E. coli* is included in the TTC analysis, similar reduction rates of this enterobacteria could be expected, which means that the estimated numbers of *E. coli* in the effluent from bark, charcoal and sand filters would have been 2×10^3- 3.6×10^4, 4.4×10^4-7×10^4 and 2×10^3- 4.2×10^4 MPN *E. coli* 100 mL^{-1}, respectively (Table 6). However, these calculated figures can be underestimates of the real numbers of *E. coli*, because it is a subgroup of the TTC. Thus it seems that none of the effluents in the present work met the Jordan standards concerning *E. coli*. However, according to WHO guidelines for reuse of greywater in agriculture, the concentrations of *E. coli* in the effluent from all filter types were within the acceptable value of 10^3-10^5 *E. coli* 100 mL^{-1} (WHO, 2006). Protection of public health is always an important issue in wastewater treatment and basing regulations on microbial indicators is constantly under debate. The presence of the indicator organisms does not necessarily imply failure, as the indicators themselves are not pathogenic. One obvious drawback in using indicator organisms is that outside their normal habitat, they are frequently observed to behave differently to the pathogens that they are intended to indicate (Leclerc *et al.*, 2001). Furthermore, indicators die off more rapidly than the pathogens or sometimes even start to grow, *i.e.* increase in numbers (Warner, 2005).

It should be pointed out that source separation of greywater from blackwater is a measure in itself to decrease health risks related to wastewater contamination. Source separation decreases pathogenic indicators by 3-4 log (Ottoson & Stenström, 2003). The risk of pathogen transmission can also be minimised substantially by applying *e.g.* localised irrigation or drip irrigation (4-6 $\log_{10}$ reduction), crop washing (1 $\log_{10}$ reduction) or cooking (6-7 $\log_{10}$ reduction) (WHO, 2006). In addition, restricting the use of treated greywater to a single household could also be a way to manage health risks. The household is probably aware of any infected family members and could therefore be more cautious in using harvested crops or even avoid using the treated greywater at all for irrigation during infection periods. However, to our knowledge, there is no reported evidence in the literature of epidemics due to use of greywater.

6.5 Recycling of spent medium

All filter materials have a finite life and their failure is a matter of time (Kropf *et al.*, 1977). Therefore, sooner or later, the spent medium will need to be replaced and the waste material will have to be disposed off. If the spent materials are to be spread onto the soil, not only should their content of carbon and plant nutrient be considered, but attention should also be paid to their hygienic and chemical quality. The possibilities of recycling spent bark and charcoal are facilitated by their light weight compared with sand, making them more advantageous as a filter material compared with sand. Using spent bark as a soil amendment should be possible and could be expected to improve the soil organic matter status (Hernández-Apaolaza *et al.*, 2005). For example, Haynes and Swift (1986) reported that soil amendment with *Pinus* bark increased both plant growth and yield of blueberry. Bark containing adsorbed NH_4^+-N and PO_4-P could probably be viewed as a slow-release fertiliser. The high water retention capacity of the bark should also enhance the water-holding capacity of the soil. Depending on the wastewater quality treated by the filter, it could also contain trapped metals, both metals essential to the plants but also potentially toxic metals. Efficient adsorption of metals using bark is well documented (Argun *et al.*, 2009; Genç-Fuhrman *et al.*, 2007; Jang, 2005). In a short-term experiment, Linde *et al.* (2007) observed increased leaching of copper and chromium from contaminated soil covered with bark and attributed this to chelates formed with dissolved organic carbon binding and transporting the metals. Leaching of trace metals and phenols has also been observed from virgin, dried and granulated pine bark (Ribé *et al.*, 2009). The advantage of charcoal for many soil functions, leading to increased yields, is well documented and includes improved soil water retention and enhanced biomass of soil biota. In addition, environmental benefits have been observed, such as reduced greenhouse gas emissions (CO_2 and N_2O) from agricultural soils and increased carbon sequestration (Lehmann *et al.*, 2011; Vaccari *et al.*, 2011; Woolf *et al.*, 2010; Deluca *et al.*, 2009). Adsorption of heavy metals to charcoal can be regarded as advantageous when it is used as a filter, but also poses a risk when the filter material is recycled. In order to avoid contamination, household members should be careful not to dispose off products containing heavy metals or other hazardous compounds in greywater. Spent sand could be spread onto agricultural fields or used as a filling material for road construction. However, excavating and transporting the spent sand may be laborious and costly due to its heavy weight.

7 Conclusions and perspectives

Bark, charcoal and sand filters were studied in order to obtain information about the performance of these materials as low-cost alternatives for on-site greywater treatment for reuse as irrigation water. Treatment requirement, medium properties, biological activities, loading conditions and handling of spent medium are core aspects in describing, understanding and evaluation of such filters. Based on the literature review and the results presented in **Papers I-V**, the following conclusions were drawn:

7.1 Greywater pollution and treatment requirement

Greywater treatment is necessary to avoid environmental, aesthetic, operational and, most importantly, public health risks. For these reasons, lowering the organic matter content in the greywater and avoiding pathogen contamination are particularly important.

7.2 Effects of medium properties

The organic composition and hydraulic properties of bark, together with its swelling potential, enhanced the ability of this material to effectively remove organic matter, suspended solids and pathogenic indicators from greywater compared with charcoal and sand. The large specific surface area of the charcoal enhanced adsorption and the capacity for biological mineralisation of organic matter and removal of phosphorus. Charcoal was the most efficient material in removing nitrogen and phosphorus. The lower effectiveness of charcoal in removing suspended solids, pathogenic indicators and tracer microorganisms, compared with the bark, was mainly due to the charcoal having more uniform macropores. The small specific surface of the sand and the low porosity did not support effective removal of organic matter.

7.3 Effects of microbial activity, diversity and composition

The nutrient content of the greywater and the microbial population and composition of the bark itself resulted in establishment of active, distinct and rich bacterial community able to effectively treat the greywater, remove organic matter and allow nitrification. The greywater was the only source of nutrients in the biologically inert charcoal and sand filters, nourishing the microbes and allowing them to form biofilm rich and diverse in bacterial community. The potential respiration activity was higher in the charcoal than in the sand. The reason for this difference is probably the large specific surface area of the charcoal, which can host and shield a larger microbial community.

The potential respiration rate, nitrification activity, bacterial diversity and composition profiles all indicated that organic matter degradation and nitrification occurred mainly in the top 20 cm of the bark and charcoal filters. Therefore, a filter depth of 60 cm for bark and charcoal seems to be redundant for these biological processes.

7.4 Effects of loading conditions and treatment capacity

The general regression models showed that pollutant reduction in all filters proved to be influenced by the hydraulic and organic loading rates not only in the present run but also by those in the previous run.

The performance of the bark filter decreased at high hydraulic loading rates, largely because high water flow rates washed out more dissolved substances from the bark. Nevertheless, the bark achieved the highest removal of easily degraded organics among the filters. In the charcoal and the sand filters, the biofilm expanded in response to increasing hydraulic loading rates, which increased the removal capacity of organic matter compared with that at lower hydraulic loading rates. The high flow velocity associated with high hydraulic loading rates in all filters accelerated the transport of phosphorus and nitrogen species, lowering their reduction rate. The charcoal filters achieved the highest removal of phosphorus and nitrogen in all hydraulic and organic loading combinations.

All filters were favoured by high loadings of organic matter, which nourished their biofilms and thereby increased the removal of organic matter, nitrogen and phosphorus. At high organic loading rates more nitrogen and phosphorus were assimilated in the biofilm. This could be considered advantageous for short-term filter performance, because it enhances the removal rate. In the longer term, however, a thicker biofilm is disadvantageous because when the organic matter load decreases this biofilm is shed, resulting in emissions of pollutants. Bark filters had the highest potential treatment capacity to deal with short term shock organic loads followed by charcoal filters.

7.5 Recycling opportunities and constraints

For the scenario of greywater recycling in rural communities in Jordan, greywater treatment in bark, charcoal and filters should provide a source of water that can be utilised for garden irrigation and can provide nitrogen and phosphorus as plant nutrients. However, the high *E. coli* levels in effluent from all filters pose a health risk in the event of direct human exposure to the treated greywater and the high nitrogen concentrations pose a risk of groundwater contamination with nitrate. Therefore to meet the Jordanian standards, measures to further decrease and shield possible pathogens and to lower levels of nitrogen are necessary.

Regarding the recycling of the spent filter materials, the spent bark can be used as a slow-release fertiliser and soil conditioner, while the charcoal can be used as a soil amendment to increase the nutrient and water-holding capacity of the soil. The spent sand might be a problem, since it has few recycling options and is difficult to handle and transport.

7.6 Future perspectives

Fungal activity might be important, particularly in the bark, for the degradation of greywater and also the bark itself. Bark and sand filters are materials obtained from nature, so archea might also play a role in the biological performance of the filters. Therefore, the domains of fungi and archea must be devoted more attention in future research to fully understand the capacity of bark, charcoal and sand filters.

Pine bark and charcoal were the filter media which performed best among the materials investigated here. However, different types and characteristics of bark and different parent materials and processing parameters of charcoal are found in different regions, so more research is needed in the future to optimise the performance of such filters.

The present study was performed in the laboratory, thus to determine the long-term treatment capacity of bark and charcoal filters needs further study in field conditions.

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